INTEGRATING TRANSPORT INFRASTRUCTURES WITH LIVING LANDSCAPES

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INTEGRATING TRANSPORT INFRASTRUCTURES WITH LIVING LANDSCAPES

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Importance of Roadless Areas for the European Conservation Network

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Protected Areas (PAs) are a main conservation tool to halt biodiversity loss. However, their performance has been often questioned and the need to improve their effectiveness is now more apparent than ever. Here, we propose Roadless Areas as a conservation target to increase the cover and effectiveness of PAs. Roadless Areas represent natural and semi-natural areas of high conservation value that have no or little traffic and provide multiple ecosystem services. Here, we develop a methodological framework to identify Roadless Areas in Europe and assess their spatial properties and conservation status. We examine how the European Union's conservation network, Natura 2000, would expand if Roadless Areas that are already partially included in Natura 2000 terrestrial sites or are adjacent to them would be added to the existing conservation network. We find that European lands are highly fragmented. Roadless Areas are unevenly distributed, and cover more than 30% of the European Union territory, with large Roadless Areas ($\geq$100 km^2) occupying about 18% of that surface. At the national level, there is a large variation in the percentage of overlap between Natura 2000 sites and Roadless Areas, with the Natura 2000 network currently encompassing between 19 and 89% of the Roadless Areas surface, depending on the member state. Our results demonstrate that Roadless Areas adjacent to Natura 2000 sites cover >65% of the total Natura 2000 surface. As Roadless Areas have limited human access, we suggest integrating Roadless Areas into biodiversity conservation networks as a timely solution to minimize conflicts over expanding PAs in the European Union and to achieve the goals of the European Union's 2020 Biodiversity Strategy.

Keywords: road-free areas, road system, green infrastructure, integrated biodiversity policy, EU 2020 biodiversity targets, protected areas

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INTRODUCTION

Despite local conservation success stories and the growing public and government interest, global biodiversity seems to be continuously in decline (Butchart et al., 2010; Pimm et al., 2014). Protected Areas (PAs) networks form the most important conservation tool to tackle this decreasing trend, yet their effectiveness has also been questioned (Rodrigues et al., 2004). The need to expand existing PAs has become an environmental issue of vital importance (Brooks et al., 2006; Watson et al., 2014), particularly under current climate change, which is expected to decrease habitat suitability for many species, including inside PAs (Araújo et al., 2011; Mazaris et al., 2013).

Over the last decades, the amount of land and sea designated as PAs has increased but their effectiveness has not been guaranteed (Pimm et al., 2014). A main obstacle to enhance the coverage and effectiveness of PAs networks reported in literature is the lack of political will (Watson et al., 2014; Kati et al., 2015). At a global scale, the location of PAs is biased toward lands with low economic value, such as areas at high altitudes, steep and remote or unsuitable for agriculture, where land conversion pressures are unlikely (Joppa and Pfaff, 2009). This location bias highlights the influence of conflicts on conservation design and planning (Margules and Pressey, 2000). A realistic and efficient solution for the expansion of the current network of PAs would require minimizing such conflicts (Grodzinska-Jurczak and Cent, 2011) and building upon existing knowledge and capacity of the established PAs (Rodrigues et al., 2004). In cases where further expansion of PAs is difficult to achieve, efforts toward enforcement of protection and effective implementation of the law should be augmented in the existing PAs (Jenkins and Joppa, 2009).

Here, we propose Roadless Areas (RAs) as a timely conservation target to increase the coverage of PAs. RAs are defined as natural and semi-natural areas where roads are absent or that have few roads with low traffic (Selva et al., 2011; van der Ree et al., 2011). They are considered as areas of high conservation value because they are relatively undisturbed by humans (Selva et al., 2011, 2015; Ibisch et al., 2016). Given that human access to RAs is limited, conflicts related to land use practices or to shifts in protection regimes are expected to be minor. The first official and successful attempt to protect RAs occurred more than 50 years ago, within the framework of the USA Wilderness Act from 1964 (Nie and Barns, 2014). Later, the RAs Conservation Rule, implemented in 2001, protected 24 million ha in the US Forest Service from further road development. The inclusion of RAs into conservation networks has been shown to enhance connectivity by acting as buffer zones to protect pristine areas, and by providing species with corridors or stopover areas during migratory and dispersal movements (Crist et al., 2005). RAs also support populations of species that have large spatial requirements and are sensitive to human disturbance (DeVelice and Martin, 2001; Torres et al., 2016). Large RAs are more resistant to invasions by exotic species, more resilient to extreme weather events, and contain more functional ecosystems than roaded areas (Selva et al., 2011, 2015; Ibisch et al., 2016). RAs provide numerous benefits to biodiversity, thus, the preservation of the remaining lands still unfragmented by roads is of particular relevance in highly fragmented continents like Europe.

The Natura 2000 is a network of PAs which forms the centerpiece of the environmental strategy in the European Union (EU) and is established under the Birds and Habitats Directives (2009/147/EC and 92/43/EC). It is implemented by each member state and often works in combination with nationally designated PAs (Evans, 2012). The Natura 2000 terrestrial component covers 18% of the EU and includes 26,533 sites across 27 countries (European Commission, 2016a). Despite the debates about Natura 2000 implementation and effectiveness (Alphandéry and Fortier, 2001; Kati et al., 2015), the establishment of the network has been shown to bring positive effects for European biodiversity and be a proper tool to deal with the biodiversity crisis (Donald et al., 2007; Gamero et al., 2016). The recent evaluation of the effectiveness of the Birds and Habitats Directives launched by the European Commission concluded that they remain relevant for tackling the key pressures on habitats and species, but there is continued need to promote solutions that optimize the accomplishment of the Directives conservation goals (European Commission, 2016b).

In this study, we explore the potential role of RAs to support the Natura 2000 network and provide quantitative information on the spatial gains of the current conservation network after a potential integration with RAs. Despite the fact that Europe contains one of the largest networks of PAs in the world and has strong environmental consciousness (Jordan, 2005), European conservation policies have not yet taken RAs into account. We quantify and assess the features of RAs in the EU and examine the spatial properties of the PAs network after the incorporation of RAs. In particular, we (a) identify terrestrial RAs and their distribution in the territory of the EU; (b) investigate how RAs are spatially distributed in relation to conservation sites of the Natura 2000 network; and, (c) design an integrated network of RAs and PAs and assess its spatial properties and benefits.

MATERIALS AND METHODS

Our analysis included the EU territory in 2013, with 27 Member States (4,290,148 km^2, **Figure 1**, Supplementary Table 1). We derived data on the spatial extent of the road network in Europe from OpenStreetMap contributors from 2014 (Geofabrik GmbH and OpenStreetMap Contributors,[1]), which is an international, non-profit foundation that provides continuously updated maps of high accuracy, generated by citizen science (Neis and Zipf, 2012). The database includes a wide range of road categories—from motorways to paths and sidewalks. The road network used in our study included eight road categories: motorway, primary, secondary, tertiary, motorway link, primary link, secondary link, and tertiary link (Supplementary Table 2). We excluded road categories that were considered as roads of low intensity or traffic (<1000 vehicles/day) following previous studies at country level in Europe (Anděl et al., 2005; Čizmić and Dragosavac, 2010; Selva et al., 2011; Department for Transport (Dft), 2016). Although these low-traffic roads could contribute to landscape fragmentation, their impact on habitat permeability, and wildlife movements is rather limited (Iuell et al., 2003; Selva et al., 2011).

To identify RAs, we established a threshold distance of 1 km from the selected road system, and thus, exclude all "roaded" areas occurring within this distance, as they represent the zones most intensively disturbed by roads. Some studies have applied smaller threshold distances to identify RAs to examine road effects on specific taxa (Forman, 1997; Eigenbrod et al., 2009).

Abbreviations: PA, protected area; RA, roadless area.

[1] Geofabrik GmbH and OpenStreetMap Contributors: Data/Maps. Available online at: http://download.geofabrik.de (accessed February 20, 2014).

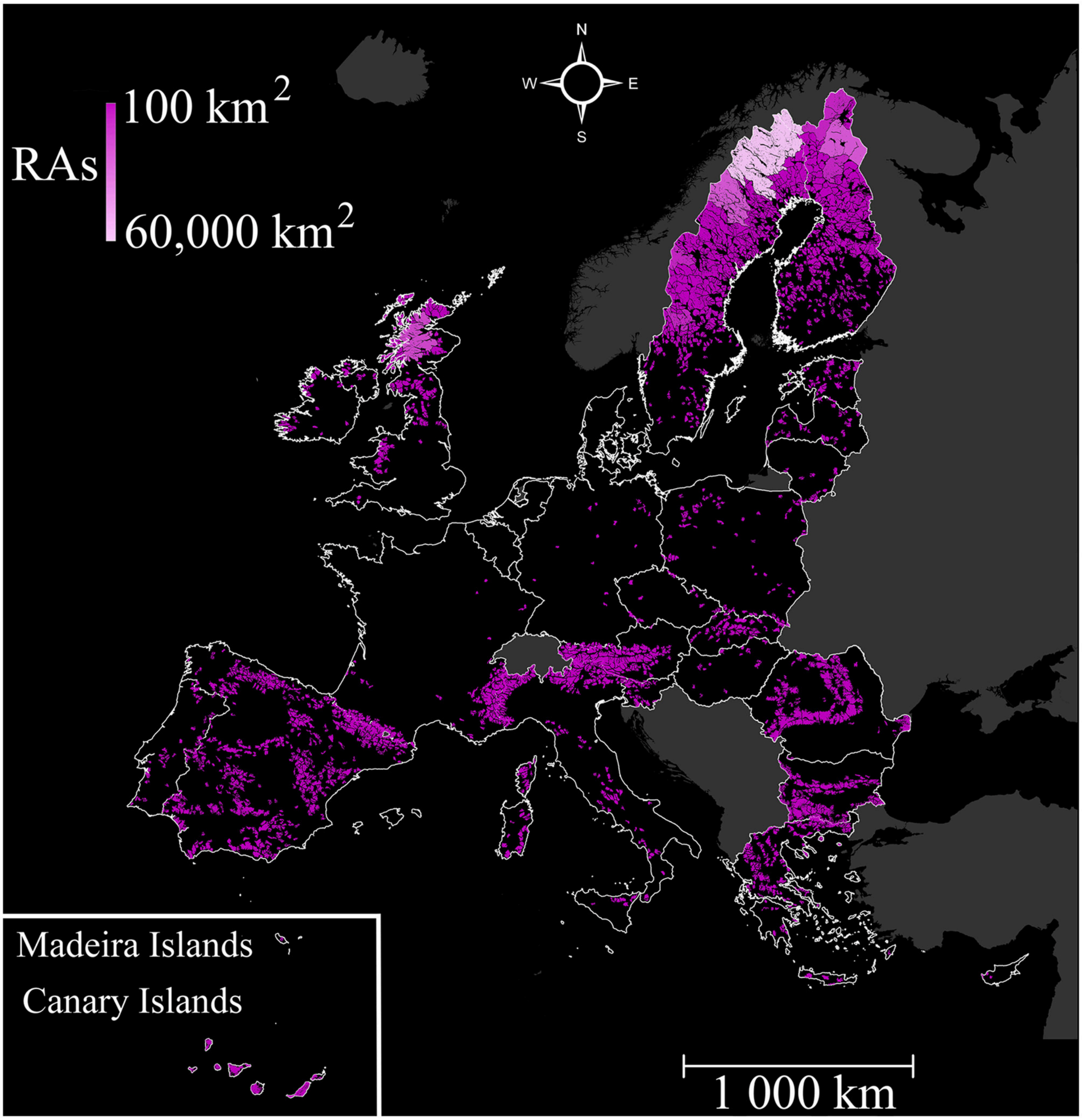

FIGURE 1 | Distribution of Roadless Areas that are larger than 100 km^2 (n = 1962 areas) in the territory of the European Union in 2014.

However, given that the spatial extent of some road impacts is of several kilometers, the distance of 1 km is suggested as appropriate for analyses at the landscape level (Benítez-López et al., 2010; Freudenberger et al., 2013; Ibisch et al., 2016).

We used the CORINE Land Cover 2000 database (Copernicus Land Monitoring, Services, 2015) to identify landscape composition. CORINE Land Cover provides spatial information for Europe's land use cover categorizing it in five main classes: artificial surfaces, agricultural areas, forests and semi-natural areas, wetlands and water bodies (European Environmental Agency, 2014). For our analysis, we only retained areas that were classified as forests and semi-natural habitats or as wetlands (Selva et al., 2011; van der Ree et al., 2015). Other land cover classes, such as large water bodies, were not included since we were interested in identifying terrestrial RAs.

Applying the 1 km buffer mentioned above, we identified a large number of road-free patches ($n = 358{,}249$; covering ≈32% of the EU surface) which fulfill the land use criteria (Supplementary Figure 1). We assess the size distribution of RAs (≥1 km^2). Then, we selected a threshold of 100 km^2 to further identify RAs large enough to individually support effective biodiversity conservation and functional ecosystems (Joppa et al., 2008; Selva et al., 2011). This threshold has already been used in similar studies in order to define road-free areas in Germany and Czech Republic (e.g., Anděl et al., 2005; Bundesamt für Naturschutz, Federal Agency for Nature Conservation, 2008; Selva et al., 2011). The distribution and size of RAs in the EU were quantified. Finally, we overlapped the maps of RAs and PAs (European Environmental Agency, 2012) to investigate the spatial relationship between RAs and the Natura 2000 sites. We calculated the surface of RAs that fell within the Natura 2000 network, and that was, therefore, protected, as well as the surface of RAs adjacent to Natura 2000 sites that could be added to the existing network of PAs (Supplementary Figure 2). All calculations were performed using GIS 10.1 (ArcGIS® software by ESRI).

RESULTS

We identified 65,000 RAs which were equal or larger than 1 km^2 (Supplementary Figure 1). However, the mean size of RAs ≥ 1 km^2 was highly variable (mean: 20.7 ± 292 km^2), and with a most frequent size of about 3 km^2 (median: 3.3 km^2). These RAs ≥ 1 km^2, cover 31% of the terrestrial surface of the EU and were found throughout the EU, with many small RAs occurring in central Europe, while large RAs were mainly located on the periphery of the EU (e.g., northern Scandinavia, northern UK, Iberian Peninsula; Supplementary Figure 3).

The number of RAs decreased exponentially with increasing patch size (**Figure 2**, Supplementary Figure 1). We found that 1962 RAs were larger than 100 km^2, covering 18.5% (793,287 km^2) of the terrestrial surface of the EU. The largest RA was found in the border region of Sweden and Finland (covering 59,225 km^2), followed by RAs occurring in northern Sweden, northern Finland, and northern UK (covering 21,410, 20,867, and 17,443 km^2, respectively). The average surface of RAs larger than 100 km^2 in the 27 studied countries was 404.3 km^2 (*SD*: ±1632 km^2). Sweden had the largest percentage of RAs in relation to its surface (55.9%), while Germany had one of the smallest percentages of RAs coverage (1.3%). We also found that the five smallest countries in Europe, namely Belgium, Denmark, Luxemburg, Malta, and the Netherlands, had no RAs larger than 100 km^2 (**Figures 1**, **3**, Supplementary Table 1). We identified a total of 174 transboundary RAs, which represented only 9% of the RAs identified, but 29% of the RAs surface (228,361 km^2). On average, transboundary RAs were larger in size (mean ± *SD*: 1312 ± 5083 km^2, median: 329 km^2) than RAs within one country.

The percentage of RAs surface that is currently protected within the Natura 2000 network is 35.5%. The degree of overlap between RAs and Natura 2000 sites highly varied across countries, ranging from 19.3% in Sweden to 89% in Cyprus. Interestingly, the three countries with the largest percentage of total RAs coverage (Sweden, Finland, and Austria) had the lowest overlap of RAs with Natura 2000 sites. These countries, along with the UK, could significantly expand their PAs network if RAs would be added (Supplementary Table 1, Supplementary Figure 4). The expansion of the network by adding adjacent RAs was minimum for Hungary and Poland (3% increase) and maximum for Sweden (310% increase; Supplementary Table 1).

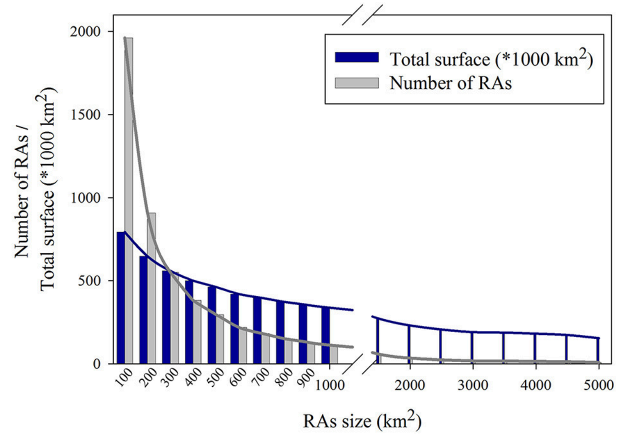

FIGURE 2 | Size distribution of Roadless Areas that are larger than 100 km^2 (n = 1962 areas) in the European Union territory in 2014.

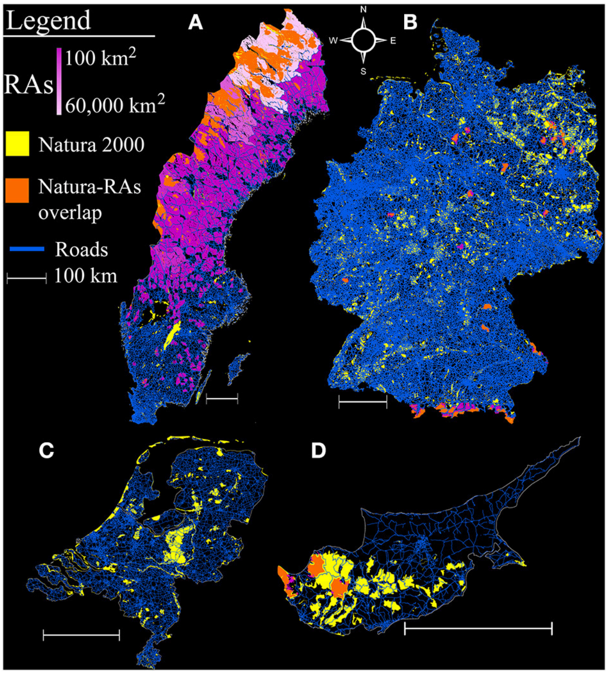

FIGURE 3 | (A–D) Distribution of the Open Street Map road network (blue), Roadless Areas larger than 100 km^2 (RAs, violet gradient) and Natura 2000 sites (yellow) in four EU countries: Sweden **(A)**, Germany **(B)**, the Netherlands **(C)**, and Cyprus **(D)**. The spatial overlap between RAs and Natura 2000 sites is indicated in orange.

In general, the Natura 2000 network could be enlarged by 66% through the inclusion of adjacent RAs; this would mean that the total area of the EU dedicated to biodiversity conservation would reach 1276 thousand km^2 then (∼30% of EU's terrestrial surface).

DISCUSSION

We proposed and tested a comprehensive framework for addressing the integration of RAs in Natura 2000, the established conservation network in the EU. First, we showed that RAs cover only 18.5% of EU territory and highly varied in size and geographical location; most roadless patches were very small in size. Second, we demonstrated that the enlargement of the EU conservation network could be achieved by designating RAs as protected sites, or by incorporating adjacent RAs into existing sites of the Natura 2000 network, which would increase the protected land up to almost 30% of EU's surface.

Transboundary regions contained many of the large RAs identified, while such large RAs are practically absent from central Europe. Though certain countries, like Germany and France, have effectively adopted EU environmental policies and fulfilled all their international nature conservation commitments (Égert, 2012), they are highly fragmented, which may compromise goals such as the ecological integrity of Natura 2000 sites (Selva et al., 2011). The intense land use and infrastructure development of most areas in central Europe since the eighteenth century is partly due to its low elevation and mild climatic conditions that have facilitated successful agriculture (Antrop, 2004). Europe is highly fragmented by transport infrastructure; half of the continent is within 1.5 km from a paved road or a railway line (Torres et al., 2016), thus, leaving not many remote areas that can be characterized as RAs (**Figures 1**, **3**, Supplementary Figure 1). Fragmentation constrains the effectiveness of EU's conservation policies (Pullin et al., 2009) and, therefore, it is important to establish and promote measures that halt fragmentation and its impacts. Such measures include bundling of transport routes and the creation of wildlife corridors (van der Ree et al., 2015), and even the implementation of a principle of No-Net-Loss of unfragmented lands as a compensation measure (Selva et al., 2015). Similar measures have been recently identified, prioritized, and adopted by the EU Green Infrastructure strategy (Lafortezza et al., 2013). Landscape fragmentation caused by transport infrastructure may be only partially mitigated, and its impacts can be entirely avoided only in areas where roads are completely absent, i.e., by the protection and restoration of RAs (Laurance et al., 2014; IENE, 2015; Ibisch et al., 2016). There are already ∼5 million km of roads in the EU (van der Ree et al., 2015), including the Trans-European Transport Network, which is planned to reach more than 170,000 km of road length by 2030 (Teodorovic and Janic, 2016; 75,000 km of roads in 2005; Tillmann, 2005). Given the magnitude of road expansion in the near future, with 25 million km of new roads in the world planned by 2050 (Laurance et al., 2014), RAs conservation should be a top priority at national, continental, and global scale (Selva et al., 2011; Laurance et al., 2014; Ibisch et al., 2016).

In many cases, PAs are located in remote or unproductive areas, which are inaccessible due to the lack of road infrastructure (Joppa and Pfaff, 2009). Their designation has prevented them from degradation caused by road impacts, thus, keeping them road-free. Nevertheless, there are cases of PAs where roads already existed and are retained to support local communities and tourism (Eagles et al., 2002). Although road development projects within the boundaries of PAs are usually limited, road removal is also an option to be seriously considered in cases of unneeded and ecologically damaging roads (Eagles et al., 2002; Selva et al., 2015). Road reclamation has an environmental impact as well; however, when combined with restoration treatments, it brings significant long-term benefits like a reduction in chronic erosion (Switalski et al., 2004). Large mammals and fish are also known to react positively to road reclamation, while vegetation and soils can develop more rapidly and sequester larger amounts of carbon when roads are decompacted during reclamation (Selva et al., 2015; van der Ree et al., 2015). Policy makers and land managers could use the results of this study to avoid further dissecting the existing RAs and determine potential targets for road optimization. Re-routing and/or removal of unneeded and ecologically damaging roads would also increase the size of RAs, and restore landscape-level connectivity. In cases of RAs which contain low-traffic roads, as the ones identified in this study, implementing speed and traffic volume limitations are also measures that could be considered in order to minimize road impacts (Selva et al., 2015).

The establishment of PAs systems aims at the conservation of species, habitats, and landscape-level processes and requires a careful identification and design process (Margules and Pressey, 2000). The designation of Natura 2000 sites is based on the presence of priority habitats and species; however, conservation targets should move from the traditional focus on species and habitats and consider more holistic approaches, which include ecosystem processes, functions, and integrity (Selva et al., 2011). In this sense, RAs represent a perfect proxy for ecosystem functionality; yet, this feature is not characteristic for most European PAs (Ibisch et al., 2016). The large variation in the percentage of RAs overlap with Natura 2000 sites across EU countries may also be indicative of the different criteria and methodological approaches applied during site designation (Palang et al., 2006).

In Europe, the designation of new PAs is needed to mitigate climate impacts to biodiversity and guarantee biological diversity persistence in the future (Araújo et al., 2011). The Habitats Directive sufficiency assessment, which was completed in 2013, concludes that not all EU member states reached their Natura 2000 targets and, therefore, further terrestrial PAs designations are needed (e.g., Cyprus, Austria, Slovakia; European Commission, 2016a). In combination with our study, this assessment could pinpoint cases where RAs prioritization could support conservation goals. For instance, the Natura 2000 network in Austria is considered to be far from complete (European Commission, 2016a), and RAs could support the Natura 2000 network's enlargement. However, in cases like Cyprus, where most RAs are already located within the Natura 2000 network (**Figure 3**, Supplementary Table 1), RAs could play only a minor role in future designations and conservation policies. Furthermore, the southern countries of the EU (e.g., Spain and Greece) are of particular conservation value, as the Mediterranean basin is a biodiversity hotspot of global importance (Myers et al., 2000), and still contain a considerable number of large RAs (**Figure 1**). In these biodiversity hotspots, RAs alone could be considered as a priority conservation target.

The integration of RAs with the Natura 2000 faces the same constraints as the establishment of any system of PAs, such as the needs of local communities (Alphandéry and Fortier, 2001) and the political will, which may not be concordant with the expansion of PAs (Grodzinska-Jurczak and Cent, 2011). However, this concept of conservation networks' enlargement has been supported by the EU 2020 biodiversity strategy, which includes for instance actions toward preserving wilderness areas (European Commission, 2011). The inclusion of wilderness areas in World Heritage Sites has also been proposed as a way to effectively protect their ecological integrity (Kormos et al, 2016). In addition, the actions included in the EU biodiversity strategy are supported by Article 10 of the Habitats Directive which calls member states to promote land-use planning and development policies that strengthen the coherence and resilience of the Natura 2000 network. Given the level of fragmentation in Europe (Torres et al., 2016; this study), RAs should be prioritized for additional protection in order to maintain landscape connectivity (Belote et al., 2016). Roadless areas, when added to existing PAs in the northern Rocky Mountains (USA), enhanced overall landscape connectivity by creating a larger and more cohesive system of PAs, by reducing isolation among PAs, and by creating a more dispersed PAs network, important for maintaining species movements (Crist et al., 2005). The conservation value of some PAs is currently at risk due to land use changes in surrounding lands (Martinuzzi et al., 2015) and protecting adjacent RAs may safeguard their values. Hence, we propose that RAs, as areas relatively undisturbed by humans, should be adopted as an alternative means of achieving the EU 2020 biodiversity strategy targets.

RAs protect biodiversity and ecosystem services, and are of particular importance in the context of climate change (Selva et al., 2011, 2015; van der Ree et al., 2015). The lack of large RAs reveals that wildlife distributions are at relatively close distances to transportation infrastructure, exposing wildlife to a number of threats (Torres et al., 2016). In this sense, mammals, in particular wide-ranging species like carnivores, would benefit from the protection and restoration of RAs (van der Ree et al., 2015; Torres et al., 2016). RAs also serve as barriers against invasive species (Strittholt and Dellasala, 2001; Selva et al., 2011), preserve the natural and semi-natural habitats of many species, and their genetic resources (Loomis and Richardson, 2000). Additionally, RAs help to stabilize the climate through carbon sequestration and nutrient cycling in forested areas (Loomis and Richardson, 2000; Selva et al., 2011, 2015) and provide multiple ecosystem services (e.g., provision of high quality water and air, erosion control, and recreational experiences; DellaSala et al., 2011). RAs sustain potential habitat suitable for a number of species and landscape-level connectivity between natural habitats, safeguarding native biodiversity (Crist et al., 2005; van der Ree et al., 2015; D'Amico et al., 2016; Torres et al., 2016).

Our study supports an innovative way to increase conservation capacity and efforts. RAs could enhance continental-scale connectivity, increasing overall coherence, and effectiveness of the Natura 2000 network. From a policy perspective, RAs represent a timely conservation tool to support EU nature conservation policies with minimum conflicts (i.e., EU 2020 Biodiversity Strategy, Green Infrastructure) and to achieve biodiversity conservation targets. Future studies should address fine or national scale assessments of RAs and their benefits to biodiversity, as well as the integration of RAs into nature conservation policy.

AUTHORS CONTRIBUTIONS

JP and AM conceived the study. NV, NS, and AM designed the analyses. MP and NV carried out data preprocessing, run the analyses, and were responsible for compiling the figures and tables. MP and NV lead writing with contributions from all authors. All five authors approve of this version to be published and agree to be accountable for this work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2017.00002/full#supplementary-material

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Road Expansion and the Fate of Africa's Tropical Forests

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The tropical forests of Africa are experiencing unprecedented changes as a result of a rapid proliferation of roads and other infrastructure. These projects are dramatically increasing access to relatively unexploited regions, particularly in the greater Congo Basin. We highlight some of the most important new projects and describe in detail an ongoing debate about a particular proposed development, the Cross River Superhighway in Nigeria. The scale and pace of new transportation projects, and the profound environmental changes they could bring, underscore a dire need for proactive land-use planning, capacity building, and environmental assessment in the nations of Equatorial Africa. It is no exaggeration to suggest that, unless carefully managed to ensure sustainability, the spate of planned and ongoing projects could irreparably diminish the forests and wildlife populations of Africa's most biologically diverse regions.

Keywords: deforestation, development corridors, equatorial Africa, infrastructure-tsunami, logging, wildlife

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INTRODUCTION

Africa's tropical forests sustain exceptionally high biodiversity and provide valuable environmental services such as hydrological functioning and carbon storage (Abernethy et al., 2016). These forests span the greater Congo Basin as well as a narrower band across the equatorial African forests. Like much of Sub-Saharan Africa, this equatorial region is facing dramatic changes in the number, extent, and environmental impacts of large-scale infrastructure projects (Laurance et al., 2015a). A particular concern is how such projects will affect important wildlife habitats, protected areas (**Figure 1**), and environmental services (Wilkie et al., 2000; Laurance et al., 2009, 2015b). Here we describe an "infrastructure tsunami" in equatorial Africa and focus in particular on Nigeria's Cross River Superhighway, a proposed project that has drawn intense scrutiny and concern. The Cross River Superhighway has drawn concern as it fails to comply with Nigerian environmental laws and does not meet international standards of good practice. For instance, the project lacks justification from a cost benefit analysis perspective (Draper et al., 2016) and has other critical deficiencies including inadequate environmental baseline data, unclear project description, insufficient identification of potential environmental impacts and potential mitigation measures, and a lack of stakeholder engagement (WCS, 2016).

Across Africa, major infrastructure projects are expanding at an unprecedented pace. These projects include a large number of industrial mining projects (Edwards et al., 2014); over 53,000 km of proposed "development corridors" (see some examples in **Figure 1**) that would crisscross much of the continent (Weng et al., 2013; Laurance et al., 2015a); the world's largest hydropower-dam complex, at Inga Falls on the Congo River; ambitious plans to increase industrial and smallholder agriculture (African Agricultural Development Company Ltd., 2013; Laurance et al., 2014a,b); widespread industrial logging (Laporte et al., 2007; Kleinschroth et al., 2015, 2016a); and a variety of other mining ventures and energy infrastructure with accompanying roads.

The planned African infrastructure expansion also threatens ecotourism revenue. For instance, approximately 62% of African forest elephants were lost between 2002 and 2011 (Maisels et al., 2013) with roads as major contributing factor (Laurance et al., 2006). It is estimated that lost economic benefit that poached elephants (both forest and savanna) would have delivered to African countries via tourism are substantial (∼USD $25 million annually) (Naidoo et al., 2016). The expected increase in African road infrastructure and therefore access to wilderness areas will continue to threaten ecotourism income including that generated by large mammals (Joseph et al., 2017).

African infrastructure expansion is being driven largely by foreign investments, most notably from China (Foster et al., 2009; IDE-JETRO, 2009; Carey and Xiaoyun, 2016), to exploit natural resources such as minerals, timber, and fossil fuels (Weng et al., 2013; Edwards et al., 2014). Another oft-cited justification is concerns about Africa's booming population—which is projected roughly to quadruple this century (U.N. Population Division, 2016). This is creating serious concerns about food-security and human-development challenges (African Agricultural Development Company Ltd., 2013; Weng et al., 2013), and broader anxieties about the potential for social and political instability.

AFRICAN DEVELOPMENT CORRIDORS

A true game-changer for African nature conservation is at least 33 ongoing and proposed "development corridors" that will crisscross sub-Saharan Africa. If completed in their entirety, the corridors would collectively

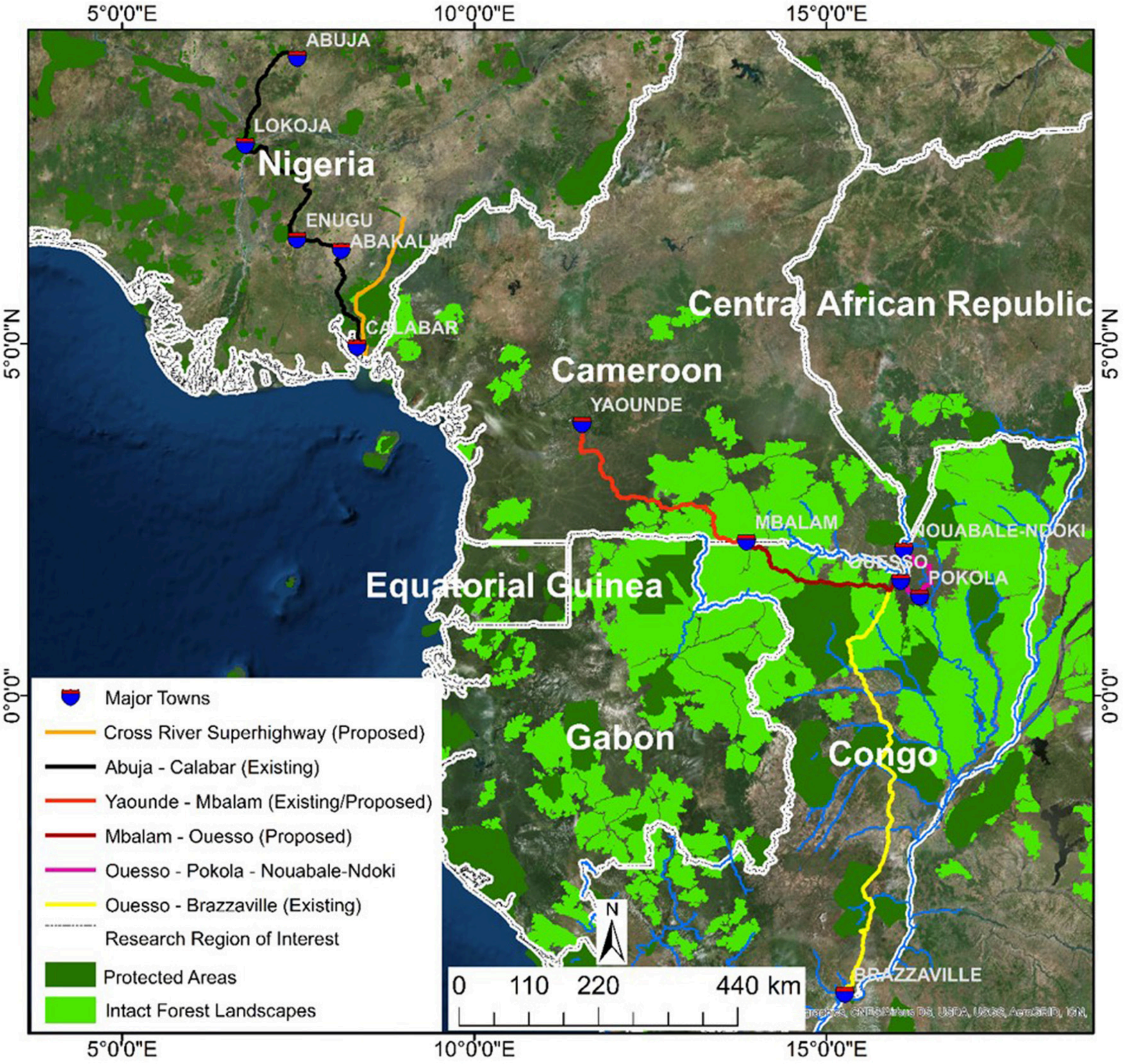

FIGURE 1 | Some of the proposed and existing highway routes in Equatorial Africa. The Cross River Superhighway in Nigeria (proposed). Yaounde-Mbalam (partly existing and proposed) Mbalam-Ouesso (proposed) and Ouesso-Brazzaville (existing). The protected areas layer was downloaded from the World Database on Protected Areas 2017 and Intact Forest Landscapes 2000 layer was acquired from the Global Forest Watch website.

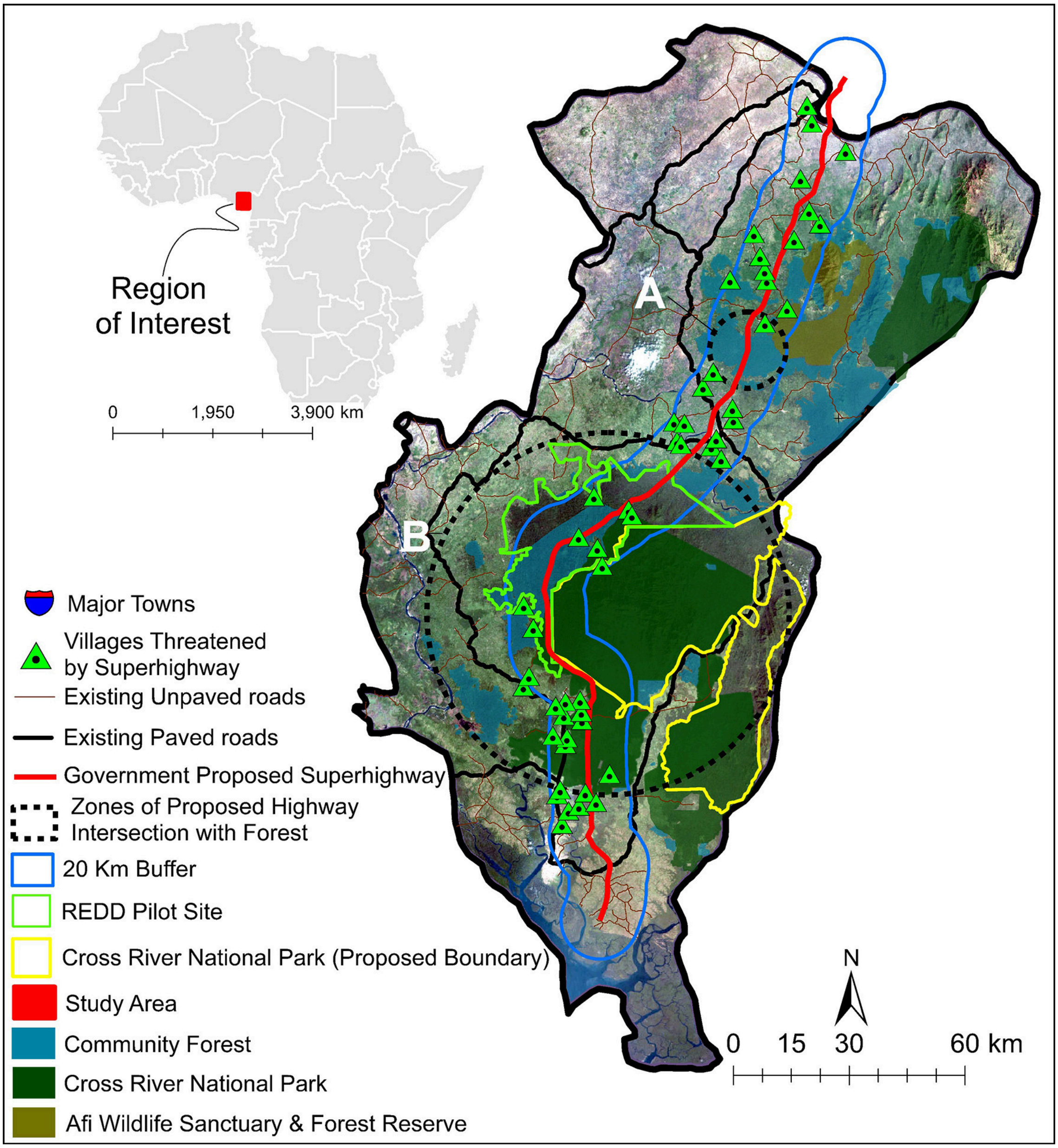

FIGURE 2 | (Top left) An inset of Africa depicting the study region. **(Right)** The proposed 260 km-long Cross River Superhighway route with its originally proposed 20 km-wide buffer zone in Cross River State, Nigeria. Circles A and B depict areas of intersection between the proposed superhighway, the Cross River national park and community forest.

span more than 53,000 km in length (Laurance et al., 2015a).

The development corridors would have a range of environmental effects, including major impacts on existing protected areas. First, they could bisect reserves, fragmenting them and opening them up to illegal encroachment, logging, mining, poaching (Jeusset et al., 2016; Sloan et al., 2016), and contagious development (Selva et al., 2015). Second, by promoting colonization, habitat loss, and intensified land uses around reserves, they could decrease the ecological connectivity of the reserves to other nearby habitats. Finally, environmental changes in the lands immediately surrounding a nature reserve tend to infiltrate inside the reserve itself (Findlay and Bourdages, 2000; Seiler, 2001; Laurance et al., 2012). So, for example, a reserve with extensive logging and hunting in its surrounding lands and weak reserve management will also tend to experience those same threats, to some degree, inside the reserve (Caro et al., 2014).

A detailed analysis of the proposed and ongoing development corridors (Laurance et al., 2015a) suggests that (1) many corridors as planned would occur in areas that have high environmental values and are only sparsely populated by people; (2) as planned, the corridors would bisect over 400 existing nature reserves; and (3) assuming that land-use changes intensify only within a 25 km-wide zone around each corridor, more than 1,800 additional reserves could experience deterioration in their ecological integrity and connectivity as well as increased human encroachment (Barber et al., 2014).

In total, the 33 development corridors could bisect or degrade about one-third of all existing protected areas in sub-Saharan Africa (Laurance et al., 2015a). Further, 23 of the corridors are still in the early planning or upgrading phases and these would be especially dangerous for nature, bisecting a larger proportion of high-priority reserves, such as World Heritage Sites, Ramsar Wetlands, and UNESCO Man and Biosphere Reserves. Collectively, the 23 planned corridors would slice through more than 3,600 km of protected-area habitat (Sloan et al., 2016).

Of the approximately 2,200 African protected areas that could be affected by planned or existing development corridors, a number are located in Central Africa. For example, two epicenters of bisected reserves—located in the mineral-rich belt spanning southern Cameroon and the northern Republic of Congo, and the Great Lakes region of East Africa—harbor vital habitats for wildlife, including great apes (Sloan et al., 2016). Considerable losses and deterioration of important habitats would also occur outside of protected areas, as human populations, agriculture, hunting, and other activities proliferate around the development corridors (Laurance et al., 2012, 2015a). The World Bank projects that, in coming decades, expanding roads and transportation infrastructure will be the biggest drivers of deforestation in the Congo Basin (Megevand, 2013).

The substantial investment in road infrastructure development planned in equatorial Africa will also significantly contribute to other underlying threats facing African tropical forests. These threats include habitat degradation and fragmentation, over-exploitation, exotic species invasion and future climate change impacts (Morris, 2010; Malhi et al., 2013, 2014). The magnitude of these impacts is not clearly known as the ecology of African tropical forests is less understood than that of the Americas and Asia (Malhi et al., 2013). This is largely due to limited scientific capacity, absence of funding for research, short-lived environmental governance plan and management regimes (Owusu et al., 2017).

THE CROSS RIVER SUPERHIGHWAY

One example of the large-scale infrastructure being planned for Central Africa is the Cross River Superhighway in Nigeria. This six lane 260 km-long highway would run from the country's far southeastern coast to Abuja, the national capital (**Figure 2**). The highway is expected to cost an estimated US$ 2.5 billion (Anonymous, 2016) and will be funded through both a Public Private Partnership and an Israeli-British firm who will contribute an estimated 500 million Euros (Akpan, 2016). As currently planned, the highway will cut through the most important forested regions in the country, a REDD pilot site and skirt the border of Cross River National Park, a proposed world heritage area (**Figure 2**). Notably, the Cross River National Park harbors the highest numbers of primate species in the world as well as Nigeria's greatest plant and animal biodiversity (Mittermeier et al., 2006; IUCN, 2010; ALERT, 2016).

Road infrastructure development such as the proposed superhighway contribute to urbanization by attracting land speculators and settlers (Anderson, 2017; Haines, 2017) which in conjunction with the highway would have devastating impacts on ecosystem functioning and threaten biodiversity (Riley et al., 2005; Pauchard et al., 2006; McKinney, 2008; Heinrichs and Pauchard, 2015). For instance, the Cross River National Park harbors roughly 20% of the planet's butterfly species which if constructed the superhighway would threaten. The superhighway would also threaten endemic species (Fahrig, 2001; Dicks et al., 2015), and contribute to the extraction of the remains of hardwood trees including the highly priced local mahogany (*Khaya snegalensis, Khaya grandifolia, and Khaya punchii*) (Thompson, 1908; Lukumbuzya and Sianga, 2017). If the highway is built along its proposed route, it would penetrate a previously roadless area of forest, allowing its exploitation and contributing to a country wide net-loss of forest (Ibisch et al., 2016).

The superhighway project has raised widespread alarm both nationally and internationally because about 90% of Nigeria's tropical rainforest has previously been destroyed and Cross River National Park sustains two-thirds of the country's remaining tropical rainforest. In this context, Cross River National Park is irreplaceable. It sustains 18 species of primates—among the highest primate diversity in the world. Among these is the critically endangered and locally endemic Cross River Gorilla (*Gorilla gorilla diehli*), which like other primates in the region is highly sensitive to hunting (Effiom et al., 2013). It also sustains high plant diversity and a variety of other imperiled wildlife species, such as forest elephants and leopards (ALERT, 2016; Ingle, 2016).

The Cross River Superhighway is in a state of flux and heated debate. Until recently, the land titles of traditional landowners were revoked by the Cross River State government within a 20 km-wide zone around the highway route, affecting at least 42 forest communities within 13 Local Government Areas, especially the Ekuri people and current REDD pilot sites. Leases to these traditional lands and wildlife habitats were reportedly going to be auctioned off by the Cross River State government, whose present governor, Ben Ayade, is a key proponent of the highway. The leases would reportedly be sold to foreign timber and mining firms, in what have been described as an abuse of Nigerian land-use laws (ALERT, 2016).

Scientists and conservationists (e.g., Abutu, 2016; ALERT, 2016; Okeke, 2016a,b; Ingle, 2016; Laurance et al., 2017) have vigorously decried the Cross River Superhighway because of its high potential to damage Nigeria's most important forest ecosystems. Already, millions of trees have been bulldozed along the proposed road route. The environmental impact assessment (EIA) conducted for the project has been widely derided, with construction of the superhighway temporarily halted by the then federal Minister for the Environment, Amina Mohammed, because of concerns about the EIA. The project is also being challenged by a lawsuit brought by nongovernmental groups in Nigeria, who claim its EIA is farcical (ALERT, 2016).

After concerted international and national pressure, the Cross River State government announced in February 2017 that it would proceed with the Superhighway, but would forego its scheme to revoke land titles and exploit lands within the 20-km wide zone along the highway route. Instead, the government claims it will appropriate lands only along the highway itself plus a forest-free zone of 70 m width along either side (totaling about 200 m width overall, including the road surface and its verges). This is clearly a positive development, but many remain concerned that the present routing of the Superhighway—running along the margin of Nigeria's most important national park and bisecting the nation's last extensive tract of tropical forest—is extremely risky from an environmental perspective. Re-routing the highway to avoid these critical habitats is a key priority.

TIP OF THE ICEBERG

The Cross River Superhighway is but one of an expanding network of large-scale development corridors in equatorial Africa. In the Congo Basin alone, the Ouesso-Bangui-N'Djamena, Libreville-Lomie, Cameroon-Chad, and Northern Upgrade Corridors will span parts of Cameroon, Gabon, Republic of Congo, Democratic Republic of Congo, and Central African Republic (Laurance et al., 2015a). At present, many forests in these nations are still remote and only partially accessible via logging roads and existing timber concessions (Laporte et al., 2007; Kleinschroth et al., 2015). Unless environmental safeguards are rapidly implemented, the spate of new infrastructure projects will open up large tracts of Central Africa to further pressures, such as mining, hunting, logging, and deforestation for agriculture (Laurance et al., 2006, 2015a; Blake et al., 2007; Poulsen et al., 2009). An urgent priority is retaining the large areas of the region affected by selective logging as native forests for biodiversity and ecosystem services, rather than seeing them being deforested or depleted of wildlife by commercial hunters and poaching (Laurance and Edwards, 2014).

Clearly, those seeking to manage the forests of equatorial Africa sustainably must grapple with serious and immediate challenges revolving around (1) effectively designing, assessing, and mitigating new infrastructure projects to limit their environmental and social impacts, (2) improving governance capacity for nations experiencing unprecedented foreign investments for infrastructure and natural-resource extraction, and (3) managing the economic and social instabilities that can plague nations largely reliant on just a few natural resources or commodities for export income—avoiding the so-called "natural-resource curse" or "Dutch Disease" (Edwards et al., 2014).

An array of solutions is needed to meet the serious challenges road developments pose with solutions varying upon the stage of road development. Prior to road construction an increased focus on proactive land-use planning (Laurance et al., 2014a, 2015a) ensuring that infrastructure locations effectively integrate sustainable agriculture (Laurance et al., 2014b) with forest conservation (Kleinschroth et al., 2016a,b) should occur. Before the commencement of an individual project, landscape level, detailed environmental and social impact assessments should be undertaken by road proponents and the resultant constraints rigorously enforced by governmental bodies (Laurance et al., 2015b). This would also allay the need for rerouting of poorly designed road projects (Mahmoud et al., 2017). Finally, the impacts of existing roads on forests could be lessened through effective forest management and law enforcement (Edwards et al., 2014; Abernethy et al., 2016). These solutions are not easily attained, but they are nonetheless urgent and essential for the future of Africa's tropical forests.

AUTHOR CONTRIBUTIONS

WL wrote the manuscript and MM, MC, and MA provided ideas, critical feedback and assisted with the revision of the manuscript.

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Birds and Roads: Reduced Transit for Smaller Species over Roads within an Urban Environment

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Roads provide humans with a means of mobility between destinations, be it for transportation of goods and services or as a means of connecting with others. However, roads are also known to contribute toward a number of deleterious landscape processes, such as habitat destruction and fragmentation, pollution (e.g., chemical, noise and light) and animal mortality. Few studies however have investigated their effects on avifauna. We investigated the influence of road width on movements of nearby bird assemblages in Australia by comparing crossing counts of four species guilds and sizes over roads of varying widths in Southern Brisbane. In addition, we also sought to determine the association of seasonality with species crossing richness and abundance. Species guild affiliations were based on definitions from previous literature: small forest dependent, large forest dependent, honeyeater, and urban tolerant; whereas body size classifications were arbitrarily defined: small (<20 cm), medium (20–29 cm), and large (>30 cm). Road sites were selected based on their vegetation likeness, both within and between sites, and this was assessed using the Specht classification system. We detected strong, negative correlations between road width and forest species richness, crossing species richness and species crossing abundance. In particular, analysis of species guild classifications revealed species richness and crossing likelihoods of the small forest dependent guild to be consistently lower than those of large forest dependent, honeyeater and urban tolerant guilds. Analysis of species by body size classification yielded similar outcomes: small birds were consistently less likely to be present nearby and cross over roads of all widths compared to medium and large birds. We believe gap permeability, particularly changes in vegetation structure and complexity that may restrict a species ability to access to the gap, as well as competition and predation, play an important role in determining species presence and crossing likelihood. Dense vegetation is known to benefit smaller species due to the provision of foraging resources and shelter from larger, more aggressive species.

Keywords: forest dependent, habitat fragmentation, road crossing, vegetation complexity, gap permeability, wildlife overpass, forest fragments, habitat connectivity

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INTRODUCTION

Although people garner a number of benefits from roads, including transportation of goods and services and connectivity, roads exert a variety of negative effects on the surrounding environment: from changes in animal and vegetation communities (e.g., fragmentation) to altered geological processes (e.g., river hydrology and run-off) (Forman et al., 2003; Zhang et al., 2013;

Morelli et al., 2014; Roberts and Sjolund, 2015; Selva et al., 2015) However, it is their ability to act as physical barriers to dispersal that has received considerable interest in the wider community, especially in instances where animals, particularly large mammals, attempt to navigate these barriers (Goosem and Marsh, 1997; Gleeson and Gleeson, 2012; Pell and Jones, 2015).

Despite their relative abundance, there have been few studies investigating the impacts of roads on birds, most likely due to the assumption that their ability to fly enables them to easily navigate fragmented landscapes and thus avoid the associated impacts (Lees and Peres, 2009; Kociolek et al., 2011; Jones and Pickvance, 2013; Kociolek et al., 2015). However, recent studies have demonstrated that birds, particularly forest dependent species, are quite susceptible to the effects of habitat fragmentation (Goosem and Marsh, 1997; Rytwinski and Fahrig, 2012; Jones and Pickvance, 2013; Evans, 2014; Pell and Jones, 2015). In fact, Lees and Peres (2009) noticed a significant number of Amazonian rainforest dependent species unwilling to cross gaps as narrow as 7 m in width, whereas Tremblay and St Clair (2009) found 45 m to be a significant threshold for dispersal.

This may be in part due to the absence of suitable habitat occurring along road verges due to road barrier and edge effects (Ford et al., 2000; Palomino and Carrascal, 2007; Evans, 2014). Habitat fragmentation is currently recognized as one of the greatest threats to species survival and is the result of sub-dividing one area of continuous habitat into smaller, separate "fragments" (Ford et al., 2000; Benitez-Lopez et al., 2010; Campbell et al., 2010; Evans, 2014). This is of particular concern as remaining habitat fragments, particularly fragment edges, become susceptible to continued disturbance through changes in abiotic (light, rainfall, resource availability, etc.) and biotic (e.g., predator-prey interaction) factors (Benitez-Lopez et al., 2010; Evans, 2014). The end result is a fragment encompassed by an "edge" that is typically intermediate in complexity to that of the fragment core and the disturbed landscape (Ford et al., 2000; Forman et al., 2003; Campbell et al., 2010; Evans, 2014). As a result, many remaining species become significantly more susceptible to population level effects (e.g., genetic drift and resource availability) as fragments become smaller and more isolated from one another (Lees and Peres, 2009; Campbell et al., 2010; Zimmer and Emlen, 2013; Zhang et al., 2013; Evans, 2014).

Traffic volume has also been shown to negatively correlate with reductions in species density and breeding (Reijnen and Foppen, 2006). Specifically, birds exposed to loud noise experienced elevated stress levels, such as increased heart rate, which over time may translate into increased risk of developing physiological stress and/or physiological disorders. Traffic noise has also been shown to interfere with bird songs, which are used in attracting mates and establishing and defending territories (Kociolek et al., 2011; Jack et al., 2015). In particular, species that utilize songs at lower frequencies were typically more adversely affected by traffic noise than those utilizing higher frequencies, leading to a net deficit of those species along road edges (St Claire, 2003; Kociolek et al., 2011).

Life history traits may also help to explain the disparities in species dispersive capabilities. Pell and Jones (2015) noticed that smaller birds, especially those dependent on forest vegetation for cover, typically displayed wide wings in proportion to their overall body size; a trait that is known to assist with short, acrobatic flight amongst dense vegetation and thus may not be suitable for extended flight (Norberg, 1989; Keast, 1996). However, not all data currently available on road crossing likelihood can be explained solely by differences in species wing morphology. According to Lees and Peres (2009), highly mobile species appear to be largely unaffected by gap distances between forest patches compared to less mobile species; the latter were rarely observed to cross wide clearings. Larger bodied birds have also been observed to cross more frequently than lighter bodied birds (Lees and Peres, 2009; Rytwinski and Fahrig, 2012). Home range also appears to be closely correlated with crossing likelihood: species present over a wider area, and thus more habitat patches, are more willing to move between forest patches than species that were more range/habitat restricted. Finally, flocking tendency in birds was found to restrict patch movement with gregarious species being much less likely to cross compared to solitary species (Lees and Peres, 2009).

In contrast, there is some evidence to suggest that some birds may benefit from roads: power-lines, signs and roadside vegetation may serve as useful ecological corridors through the provision of suitable nesting, refuge and perching habitats (Morelli et al., 2014). Road surfaces and verges may also act as potential foraging and scavenging sites and may even aid species in energy conservation (i.e., core body temperature) (Laurance, 2015). The provision of roadside lighting may also extend foraging time and activities of both diurnal species, being able to forage for longer hours each day, and nocturnal species, using the lights to hunt for congregating prey items (Morelli et al., 2014). However, only certain species appear to benefit from such structures; these include raptors and other scavenging species, urban adapted, introduced and woodland species (Morelli et al., 2014). For other taxa, it is suggested that these same features further amplify the effect of road mortality through collisions with vehicles (Orlowski, 2008; Benitez-Lopez et al., 2010; Kociolek et al., 2011; Jack et al., 2015).

Nevertheless, birds form part of an intricate web within the environment, providing and maintaining numerous ecosystem services that humans are dependent on for sustained growth and prosperity (Whelen et al., 2008; Campbell et al., 2010). For example, birds are important predators of agriculturally important pest species (Whelen et al., 2008; Philpott et al., 2009), pollinators and dispersers of medically and economically important plant species (Wenny et al., 2011) and ecosystem mediators (Anderson, 2003). This is of particular concern in urbanizing areas where habitat destruction and roads increasingly fragment the landscape. In southeast Queensland, Australia, for example, ∼273 species (30% of all Australian species) are currently distributed across the highly urbanized Bulimba Creek catchment, nearly 50% of which occur in three or fewer isolated populations (Evans, 2014). Moreover, the demand for more and better roads continues to rise as the number of people living and working within southeast Queensland increases, which will in turn further fragment animal populations in an already highly urbanized landscape (Jones et al., 2011; Zhang et al., 2013; Evans, 2014; Jones et al., 2014; Jack et al., 2015).

It is therefore important to better understand the relationship between birds and roads in Southeast Queensland so that susceptible species can be identified and appropriate corrective measures or mitigating strategies can be incorporated into future planning.

The overall aim of this study was to investigate the relationship between road width and the associated probability of birds crossing them in Southern Brisbane. Specifically, this paper attempted to address the following questions:

(A) Do roads of different widths influence forest species presence adjacent to roads in Southern Brisbane?
(B) Does road width influence species crossing preferences based on guild and body size classifications?
(C) Does roadside species composition differ to forest species composition based on guild and body size classifications?

It is hoped that the findings will further improve upon current knowledge and understanding of fauna movements and roads as well as highlight the need for funding for artificial animal crossings to help circumvent road crossing mortality rates and maintain populations within a fragmented landscape.

METHODOLOGY

This study took place within the southern suburbs of Brisbane, a city of approximately two million people located in coastal south-east Queensland, Australia. Monitoring was undertaken in 12 study sites along roads of varying width: four small (15 m), four medium (∼40 m), and four large (∼75 m), each comparable to one to two-lane, four-lane and six-lane carriageways, respectively. Each section examined was 30 m in length and this was to ensure accurate identification of crossing species. Study sites were selected based on vegetation likeness, that is, roads with similar vegetation on both sides, as it was assumed that bird crossings were more likely to occur between similar habitats than dissimilar habitats. It should be noted that the terminology of a road gap within this paper refers to the widths of the sealed surface as defined as small (15 m), medium (∼40 m) or large (∼75 m). GPS coordinates of all survey sites were recorded using a Garmin eTrex Venture personal navigator and were accurate to within 11 meters.

Site Descriptions and Vegetation Assessment

For the purposes of this investigation, vegetation structure and composition of forest sites were classified using the standardized Specht "Vegetation Classification by Structure" (Specht, 1970; Australian National Botanic Gardens, 2014). For site details, see Appendix 1 in Supplementary Material.

Bird Crossing Surveys

Overall, the monitoring method was adapted from that of a similar, previously conducted study (Pell and Jones, 2015). Three surveys were conducted at each road site; one road survey and two forest surveys.

Road surveys were conducted by a single observer along the road edge at each site over a 20-min period. Prior to the commencement of the study, a 30 m wide count area was established, using marking tape, at each of the road sites. From the edge, the observer was then stationed in the middle of each count area and recorded any birds that successfully flew across the road. For the purposes of this study, a successful crossing was defined as an event in which the individual(s) traveled from one side of the road to the other, either landing within the road-side vegetation or continue through. Individuals observed to either enter or exit the count area during their fly-over were also recorded as having successfully crossed. For example, a bird was recorded as having crossed if it entered the count area but exited away from and outside of the count area on the opposite side and vice versa. Individuals that either crossed away from the count area or did not cross to the opposite side (i.e., they returned or flew along the road) were not recorded as having completed a successful crossing.

Forest surveys were conducted in tandem to the road surveys. For the purposes of this study, forested sites surveyed within urban areas were a minimum of 30 ha in size and contiguous with surrounding forest fragments via a wildlife corridor network. During these, the observer would stand at a point within the roadside vegetation, ∼100 m in from the road edge, and record all new observations and vocalizations over a 20 min period. It should be noted that a species did not necessarily need to be sighted in order to be recorded as being present within the forest survey site. All records were identified to the species if possible through the use of the field guide Morcombe (2004). Two forest surveys were conducted at each site on opposite sides of the roads.

The first site survey began ∼30 min after sunrise. A maximum of three road sites were surveyed each morning, with a total of nine surveys completed between 06:30 and 10:00 during the spring 2015 period (September-November 2015) and 5:30 and 9:30 during the summer 2015/2016 period (December-February). All 15 study sites were surveyed four times each during spring (September-November 2015) and four times each during summer (2015 December-February 2016). Data obtained from road and forest surveys were later combined to create a master species list specific to each road site.

All necessary permissions were obtained and the appropriate authorities notified prior to beginning all road survey work. At no point did the observer enter the road, other than to cross from one side to the other to reach the next survey site and only if and when safe to do so. Permissions were also sought prior to accessing and conducting survey work on private property.

Species Classifications

Species guild classifications were based on Pell and Jones (2015) and were as follows:

- Urban tolerant–species that are generalist species capable of colonizing and persisting within a range of different environments, although are typically associated with humans and related activities.
- Honeyeater–small to medium sized birds typically reliant on flowering trees for food and may travel great distances in search of blooms.

- Large forest dependent–birds >40 g in weight and are generally found within forest and woodland. Diets are species-specific, but commonly consist of insects (insectivores), seeds (granivores) and fruit (frugivores), although some larger species may incorporate small animals into their diets (omnivores).
- Small forest dependent–birds <40 g in weight and are typically reliant on forest vegetation. Diets tend to consist of small insects, seeds and fruit.

Species body size was also investigated and analyzed in tandem with species guild classifications. Body size was investigated as an alternative to species guilds: previous literature has focussed primarily on investigating the relationship between species life histories (i.e., guilds) and level of susceptibility to road barrier effects. Species body size classifications are as follows:

- Small: birds <20 cm in length,
- Medium: birds 20–29 cm in length,
- Large: birds ≤ 30 cm in length.

Statistical Analysis

The numbers of species and species types present, species and individuals crossing and crossing times were recorded. Count gathered data were used to calculate rates of crossing and probabilities at the species guild and body size levels. Particular emphasis was placed on differences in species presence at forest and road crossing sites, as well as numbers of crossing individuals of species of different guilds and body sizes. The outcomes of species guild and species size richness, crossing counts across the three road gap categories (small, medium and large) were analyzed using negative binomial regression. The relationship between forest species richness and road crossing counts was examined using bivariate correlation. The degrees of similarity between study sites during and between spring and summer were assessed by lower triangular resemblance matrices, using Sorensen Index of Similarity (Dice Indicies), and Multi-Dimensional Ordnance. All statistical analyses were conducted using IBM Statistical Package for the Social Sciences (SPSS) Statistics software (Version 22.0. Armonk, NY: IBM Corporation) and STATA (version 14.0, College Station, TX: StataCorp LP, USA). $P < 0.05$ were considered statistically significant.

RESULTS

Surveys of species forest and road gap richness at small, medium and large roads were undertaken between spring 2015 (September-November) and Summer 2015/2016 (December-February). In total, 88 species were recorded at forest survey sites during the 6-month observation period: 24 small forest dependent, 24 large forest dependent, 11 honeyeater and 29 urban tolerant. A total of 770 birds (from 51 species) were observed to cross roads during the 6-month observation period: 88 small forest dependent (from 10 species), 129 large forest dependent (from 9 species), 117 honeyeater (from 11 species), and 376 urban tolerant (from 21 species).

Site Vegetation

Similar vegetation was observed across all 15 study sites and was consistent with the Specht description of open forest: projective cover of the tallest stratum between 30 and 70% and tallest stratum between 10 and 30 m (Appendix 2 in Supplementary Material). Although canopy stratum remained similar across the study sites, differences in structure and composition of the understorey layers were observed: understorey cover was visibly denser adjacent to small gaps, whereas this was largely diminished nearby large gaps.

Birds Present at Forest Sites

Compared with small roads, no significant difference was observed in forest species richness for either medium roads (incidence rate ratio [IRR] 1.08, 95% confidence interval [CI] 0.91–1.28, $p = 0.41$) or large roads (IRR 0.88, 95% CI 0.74–1.06, $p = 0.18$) (overall $p = 0.10$). However, when spring counts were analyzed separately, forest species richness near large roads was significantly lower than both small roads (IRR 0.73, 95% CI 0.57–0.95, $p = 0.02$) and medium roads (IRR 0.75, 95% CI 0.58–0.97, $p = 0.03$). For summer, no differences were observed in forest species richness between small, medium and large roads (overall $p = 0.37$).

Species Guilds and Forest Species Richness

Following adjustment for species guilds, forest species richness adjacent to large roads tended to be lower than that adjacent to small roads (IRR 0.88, 95% CI 0.74–1.06, $p = 0.17$) and was significantly lower than that adjacent to medium roads (IRR 0.82, 95% CI 0.69–0.98, $p = 0.03$) (**Table 1**). The reduction in forest species richness adjacent to large roads was significantly more apparent during spring (small roads reference; medium roads IRR 0.98, 95% CI 0.98, 95% CI 0.77–1.24, $p = 0.86$; large roads IRR 0.74, 95% CI 0.57–0.95, $p = 0.02$) (overall $p = 0.04$). Forest species richness adjacent to large roads was also significantly lower than that for medium roads (IRR 0.76, 95% CI 0.58–0.98, $p = 0.03$). No such differences were observed during summer (overall $p = 0.32$).

Compared with the small forest dependent guild, species crossing richness was significantly lower for both the large forest dependent guild (IRR 0.74, 95% CI 0.6–0.92, $p = 0.01$) and the honeyeater guild (IRR 0.66, 95% CI 0.53–0.82, $p < 0.01$), but higher for the urban tolerant guild (IRR1 0.50, 95% CI 1.25–80, $p < 0.01$) (overall $p < 0.001$). Similar findings were apparent

TABLE 1 | Overall species richness at forest sites arranged by species guilds.

Road width	Small forest dependent	Large forest dependent	Honeyeater	Urban tolerant
Small	20	18	9	23
Medium	23	18	10	23
Large*	19	15	8	24

**Denotes significantly different result from either one or both other road width types within the same species category.*

during both spring (small forest dependent reference; large forest dependent IRR 0.67, 95% CI 0.49–0.91, $p = 0.01$; honeyeater IRR 0.69, 95% CI 0.50–0.94, $p = 0.02$; urban tolerant IRR 1.43, 95% CI 1.11–1.9, $p = 0.006$) (overall $p < 0.01$) and summer (small forest dependent reference; large forest dependent IRR 0.82, 95% CI 0.61–1.12, $p = 0.19$; honeyeater IRR 0.63, 95% CI 0.45–0.87, $p = 0.01$; urban tolerant IRR 1.56, 95% CI 1.21–2.03, $p < 0.01$) (overall $p < 0.01$).

Species Body Size and Forest Species Richness

Following adjustment for species body size, forest species richness adjacent to large roads tended to be lower than that adjacent to small roads (IRR 0.88, 95% CI 0.74–1.06, $p = 0.18$) and was significantly lower than that adjacent to medium roads (IRR 0.82, 95% CI 0.70–0.98, $p = 0.03$) (**Table 2**). The reduction in forest species richness adjacent to large roads was significantly more apparent during spring (small roads reference; medium roads IRR 0.94, 95% CI 0.77–1.24, $p = 0.856$; large roads IRR 0.73, 95% CI 0.57–0.95, $p = 0.02$) (overall $p = 0.03$). Forest species richness adjacent to large roads was also significantly lower than that for medium roads (IRR 0.75, 95% CI 0.58–0.97, $p = 0.03$). No such differences were observed during summer (overall $p = 0.37$).

No differences were observed between different body size groups (overall $p = 0.33$).

Which Birds Crossed Roads?

Road Crossing Species Richness

Large roads were associated with significantly lower species crossing richness compared with both small roads (IRR 0.45, 95% CI 0.29–0.70, $p < 0.01$) and medium roads (IRR 0.40, 95% CI 0.25–0.61, $p < 0.01$) (overall $p < 0.001$). The reduction in crossing richness was similarly apparent during both spring (small roads reference; medium roads IRR 1.07, 95% CI 0.68–1.69, $p = 0.761$; large roads IRR 0.39, 95% CI 0.21–0.71, $p < 0.01$) (overall $p = 0.002$). and summer (small roads reference; medium roads IRR 1.2, 95% CI 0.67–2.14, $p = 0.54$; large roads IRR 0.51, 95% CI 0.26–1.01, $p = 0.05$) (overall $p = 0.04$). Equivalent results were observed even after multivariable adjustment for species guild and body size (**Tables 1**, **2**).

Species guild

Following adjustment for road size, road crossing species richness also differed significantly between species guilds (overall $p < 0.0001$) (**Table 3**). Compared with the small forest dependent guild, crossing species was higher for all other guilds (large forest dependent IRR 1.62, 95% CI 0.99–2.63, $p = 0.06$; honeyeater IRR 1.69, 95% CI 1.04–2.75, $p = 0.03$; urban tolerant IRR 3.31, 95% CI 2.13–5.13, $p < 0.0001$). Similar patterns were observed during both spring (overall $p = 0.005$) and summer ($p < 0.001$). Sorenson Index of Similarity revealed relatively uniform species distributions at all study sites during spring and summer.

TABLE 2 | Overall species richness at forest sites arranged by body size.

Road width	Small bird	Medium bird	Large bird
Small	17	13	20
Medium	21	14	20
Large*	16	11	19

**Denotes significantly different result from either one or both other road width types within the same species category.*

Pecies body size

Following adjustment for road size, road crossing species richness was significantly greater for both medium birds (IRR 2.02,95% CI 1.37–2.97, $p < 0.001$) and large birds (IRR 1.58, 95% CI 1.06–2.37, $p = 0.03$) compared with small birds (overall $p = 0.002$) (**Table 4**). Although a similar pattern was observed during summer (overall $p < 0.001$), no significant differences were observed between small, medium and large birds during spring (overall $p = 0.39$).

Numbers of Birds Crossing Roads

Overall, large roads were associated with significantly lower road crossing counts compared with medium roads (IRR 0.47, 95% CI 0.25–0.88, $p = 0.02$) and tended to be associated with lower counts compared to small roads (IRR 0.60, 95% CI 0.32–1.12, $p = 0.11$) (overall $p = 0.05$). Similar results were observed during spring (overall $p = 0.03$), although no differences were observed between road sizes during summer (overall $p = 0.55$).

Species guilds and numbers of birds crossing roads

Following adjustment for species guilds, overall road-crossing counts were not significantly different between

TABLE 3 | Total number of species observed to cross roads arranged by guild membership.

Species category	Road width					
	Spring			Summer		
	Small	Medium	Large*	Small	Medium	Large*
Small forest dependent*	5	2	0	5	5	1
Large forest dependent	4	7	4	6	5	2
Honeyeater	8	8	1	5	7	2
Urban tolerant	8	14	6	8	10	7
Total	25	31	11*	24	27	12*

**Denotes significantly different result from either one or both other road width types within the same species category.*

TABLE 4 | Total species observed to cross roads arranged by body size.

Body size	Road width					
	Spring			Summer		
	Small	Medium	Large	Small	Medium	Large*
Small*	9	6	0	7	7	1
Medium	7	12	5	12	12	4
Large	9	13	6	5	8	7
Total species crossed	25	31	11*	24	27	12*

**Denotes significantly different result from either one or both other road width types within the same species category.*

different road sizes (overall $p = 0.14$) and during spring ($p = 0.12$).

However, a significant difference was found between different guilds (overall $p < 0.001$) in that the urban tolerant guild had significantly higher crossing counts than the small forest dependent guild (IRR 3.07, 95% CI 1.73–5.47, $p < 0.001$). A similar pattern was observed during summer (overall $p < 0.001$), but not during spring (overall $p = 0.21$).

Species body size and numbers of birds crossing roads

Following adjustment for body size, large roads were associated with significantly lower road crossing counts compared with both medium roads (IRR 0.45, 95% CI 0.26–0.79, $p = 0.005$) and small roads (IRR 0.41, 95% CI 0.22–0.75, $p = 0.004$) (overall $p = 0.005$). Similar results were observed during spring (overall $p = 0.007$), although no differences were observed between road sizes during summer (overall $p = 0.22$).

Compared with small birds, medium birds crossed roads more frequently (IRR 3.48, 95% CI 1.93–6.27, $p < 0.001$), although large birds did not (IRR 1.44, 95% CI 0.79–2.62, $p = 0.23$) (overall $p < 0.001$). Similar results were observed during both spring (overall $p = 0.008$) and summer ($p = 0.006$).

How Many Forest Bird Species Crossed Roads?

During both survey periods, a total of 88 species was recorded at forest survey sites, 51 (58%) of which were observed to undertake successful road crossings (**Table 5**). A moderately strong positive correlation was observed between forest species richness and road crossing species richness ($r = 0.43$, $p < 0.0001$).

TABLE 5 | Number of species in each guild detected at forest sites over the duration of the study.

	Species guild	Forest richness	Crossed roads
Total	Small forest dependent	24	10
	Large forest dependent	24	11
	Honeyeater	11	9
	Urban tolerant	29	21
Spring 2015	Small forest dependent	22	6
	Large forest dependent	21	8
	Honeyeater	9	9
	Urban tolerant	26	17
Summer 2015/2016	Small forest dependent	23	7
	Large forest dependent	19	9
	Honeyeater	10	7
	Urban tolerant	24	15

DISCUSSION

Key Findings

The primary objective of this investigation was to examine the road crossing likelihoods of birds present in Southern Brisbane in the context of growing levels of habitat fragmentation and urbanization. Despite the significance of these issues, as well as strong interest within the wider community, few studies have investigated gap-crossing abilities of these species. Those that have taken place have occurred in very different regions and outside of urban settings (St Claire, 2003; Lees and Peres, 2009; Benitez-Lopez et al., 2010; Kociolek et al., 2011).

The present study demonstrated that large roads were independently associated with reduced bird crossing counts and species cross richness compared with medium and small roads. Subsequently, these findings were further enhanced when adjusting for the influence of species guilds and species body size. Although it was hypothesized that all four species guilds would be similarly affected by road width, the present study revealed the small forest dependent guild to be the most prone: species richness at forest sites and crossing over road sites (i.e., number of species crossing) was consistently lower compared to the large forest dependent, honeyeater and urban tolerant guilds. This finding was partly consistent with a number of previous studies (St Claire, 2003; Reijnen and Foppen, 2006; Lees and Peres, 2009; Kociolek et al., 2011; Pell and Jones, 2015) and was further reinforced by the analysis of species body size, which revealed that "small" (<20 cm) birds were consistently less likely to cross roads compared to medium (20–29 cm) and large birds (>30 cm).

Similar observations were also made in one other prior study (Pell and Jones, 2015) examining the relationship between urban roads and bird crossings in Australia. Specifically, Pell and Jones (2015) observed reductions in species richness and numbers of birds crossing over roads, especially those of small forest dependent species. Pell and Jones (2015) also noted several species unwilling to cross directly over a main road, instead crossing via a nearby vegetated fauna overpass. However, the primary focus of their study was to assess the conservation value of fauna overpasses in movement solutions. The present study builds on the findings by Pell and Jones (2015) by (a) providing statistically more powerful and generalizable results with respect to bird-gap crossings (12 sites across the southern Brisbane region vs. 4 sites near Karawatha Forest, respectively); (b) accounting for seasonal variation in species movements; and (c) comparing and contrasting the influence of both individual size and species guild classification on species road gap crossing likelihood.

Habitat Augmentation

Previous studies have highlighted the importance of the road barrier effect in determining the distributions of numerous fauna and flora species near roads and related structures (Forman et al., 2003; Morelli et al., 2014; Van der Ree et al., 2015). Birds have been identified as being highly susceptible to road barrier effects despite their ability to fly (Morelli et al., 2014), with fewer species being present near roads, a finding reflected in the results of the current study.

Roadside vegetation, particularly the understory, was observed to diminish with increasing road width and this coincided with reductions in both species forest richness and road crossing likelihood, especially those of smaller species. In particular, wider roads (e.g., Logan and Pacific

Motorways) appeared more highly disturbed, with visibly sparser vegetation and wide maintained grass "shoulders"; whereas small roads (e.g., Hemmant-Tingalpa Road, Pine Mountain Road) appeared to be more "intact," with denser vegetation nearer to the road. Importantly, numerous studies have reported similar associations between crossing likelihood and vegetation structure (Erickson et al., 2005; Orlowski, 2008; Lees and Peres, 2009; Thinh et al., 2012; Jones and Pickvance, 2013; Laurance, 2015).

Habitat destruction and fragmentation often result following the construction of roads, which in turn lead to altered environment conditions (often negative) in the surrounding landscape (Ford et al., 2000; Kociolek et al., 2011; Amos et al., 2014). This is believed to directly impact upon nearby populations of small forest dependent birds due to their heavy reliance on resources supplied by the dense understory and mid-story vegetation layers (i.e., food, shelter and nesting sites) (Desrochers and Hannon, 1997; Ford et al., 2000; Kutt and Woinarski, 2007; Tremblay and St Clair, 2009; Jones and Bond, 2010; Jones et al., 2011; Thinh et al., 2012; Laurance, 2015). Indeed, Kutt and Martin (2010) observed foraging height to accurately predict species responses to changing native vegetation structure and complexity in north Australian savannah woodlands. Specifically, small insectivores (e.g., rufous whistler, *Pachycephala rufiventris*; gray fantail, *Rhipidura fuliginosa*; and variegated fairy-wren, *Malurus lamberti*) were found to be most susceptible to changes in forest structure and complexity, whereas species foraging within the upper strata, such as honeyeaters (e.g., noisy friarbird, *Philemon corniculatus*; and little friarbird, *Philemon citrogularis*) and large insectivores (e.g., black-faced cuckoo-shrike, *Coracina novaehollandiae*; and gray butcherbird, *Cracticus torquatus*), were less susceptible (Kutt and Martin, 2010). This is significant as many forests within Brisbane, specifically public bushland and reserves adjacent to built-up areas, are exposed to multiple maintenance regimens, including fire, mowing and spraying.

This may further exacerbate the deleterious influence of roads for a number of nearby populations through the establishment of highly competitive edge specialist species capable of utilizing the new resources provisioned by roads (Grarock et al., 2014; Morelli et al., 2014). For example, a number of highly aggressive species, including the noisy miner (*Manorina melanocephala*), blue-faced honeyeater (*Entomyzon cyanotis*) and noisy friarbird (*Philemon corniculatus*), were regularly observed at a number of more highly disturbed sites in the current study (personal observation). Previous studies have observed sharp declines in the richness of smaller birds, including other honeyeaters, in resource scarce areas where they were present at moderate numbers (Ford et al., 2000; Oldland et al., 2009; Kutt et al., 2011; Montague-Drake et al., 2011). Furthermore, gaps in vegetation may also serve as territory boundaries for a number of edge specialist species that may further restrict the home-range gap crossings of conspecifics (Lees and Peres, 2009). For example, in the present study, several sacred kingfisher (*Todiramphus sanctus*) pairs were recorded near small roads and associated structures but were rarely observed to cross (CJ personal observation). On the few occasions that this happened, brief but aggressive fights ensued (CJ personal observation).

Similarly, small birds are highly susceptible to predation outside of cover: observations of many large predatory species within this study appeared to coincide with visible reductions in species movements and activities, particularly those of smaller birds, a finding consistent with those of previous studies (Desrochers and Hannon, 1997; Orlowski, 2008; Lees and Peres, 2009). Moreover, Jacobson et al. (2016) proposed a species susceptibility to road barrier effects to be related to their ability to recognize and respond to potential predators and threats. "Nonresponders" failed to recognize the threat and crossed irrespective of traffic conditions; "Pausers" stopped in the face of danger; "Speeders" fled in the face of danger; while "Avoiders" were able to recognize traffic as potential predators and began to avoid roads at lower traffic volumes. Within the current study, small forest dependent species were more often observed within forest sites nearby roads, with noticeably few observed to cross. On the other hand, large forest dependent, honeyeater and urban tolerant species appeared less deterred by road gap width.

Species Morphology

Both Lees and Peres (2009) and Jones and Pickvance (2013) noted that disproportionate numbers of larger bodied species crossed open gaps in vegetation between fragments, whereas smaller bodied species appeared to prefer vegetated corridors when crossing. This may reflect differences in species life histories (Rytwinski and Fahrig, 2012). For example, Keast (1996) found wing-shape to vary significantly with species habitat preferences. Specifically, small rainforest species possessed wider and rounded wings, a configuration suited to maneuvering amongst dense vegetation (e.g., understory) and capturing insects, whereas larger eucalypt woodland species possessed long and narrow wings for strong and extended flight over distances (Norberg, 1989; Keast, 1996). This may therefore mean that small forest dependent species in general are ill equipped to cross larger roads, as observed in this study. However, it should also be noted that this wing-body morphology is also expressed in some larger species, such as the pheasant coucal and brush-turkey, both of which exhibited similar trends in crossing likelihood.

Traffic

Habitat degradation is known to reduce the surrounding landscape's ability to buffer against noise pollution, particularly within forested regions (Reijnen and Foppen, 2006; Benitez-Lopez et al., 2010). Previous studies have shown several species to experience elevated levels of stress, including elevated heart rate and hypervigilance (i.e., predator awareness), following short-term exposure to loud noises associated with high vehicle volume roads, prompting many to retreat to quieter areas further away (St Claire, 2003; Reijnen and Foppen, 2006). Helb and Hupop (in Reijnen and Foppen, 2006) suggest that repeated long-term exposure to such noise levels may increase risk of physiological and psychological stress and disorders.

Artificial noise, such as that produced by traffic, may also mask bird calls of similar frequencies (Rheindt, 2003; Goodwin and Shriver, 2010; Francis et al., 2011). This is especially important as birds, particularly passerines, use calls in communication, territory defense and courtship (St Claire, 2003; Reijnen and Foppen, 2006; Read et al., 2015). In a recent study, Read et al. (2015) observed disturbance responses of 89 Australian species at varying distances from a nearby mine (Olympic Dam Mine) for potential use as indicators of environmental health. Unsurprisingly, fewer species were present at sites nearer the mine compared to sites situated further away, which the authors attributed to masking by noise produced by the mine (Read et al., 2015). However, acoustic masking is thought to primarily affect species utilizing low pitch calls, a trait typically associated with larger-bodied birds. This may in part explain why fewer observations were made in forests nearer wider, high traffic roads for some large forest dependent and urban tolerant species (e.g., gray shrike-thrush, *Colluricincla harmonica*; pheasant coucal, *Centropus phasianinus*; eastern koel, *Eudynamys scolopacea*; pied butcherbird, *Cracticus nigrogularis*; and willie wagtail, *Rhipidura leucaphrys*). However, it should be noted that although previous studies have identified negative correlations between traffic noise and species richness and abundance, traffic noise was not the dominant cause for the declines (Summers et al., 2011).

Seasonal Variation: Forest Richness and Road Crossings

The findings of this study suggested modest seasonal fluctuations in species abundance. Species richness across the forest sites appeared to become more uniform during summer. Similarly, numbers of species observed to cross increased slightly during summer. Sorenson's Index of Similarity also revealed slight increases in species compositions at forest sites and crossing roads during summer.

It is quite possible that these observed patterns may relate to seasonal variations in resource availability. According to Reside et al. (2010), rainfall within Australia is highly seasonal due to strongly variable inter-annual weather patterns, which in turn influences the abundances of flowering plants and insects. As a result, a number of species, most notably the honeyeaters, have evolved high vagility in order to track these episodic resource booms (Griffioen and Clarke, 2002; Reside et al., 2010). In particular, noticeably fewer observations of a number of species, such as scarlet honeyeater, brown honeyeater, white-throated treecreeper, mistletoebird, and gray fantail, appeared to coincide with lower perceived rainfall frequency and bloom intensity at forest sites during summer (CJ personal observation). However, this explanation does not support the observed increase in species forest and road compositions between seasons, possibly due to greater richness of a number of species normally considered sedentary (Griffioen and Clarke, 2002).

Alternatively, seasonal patterns in the data may be more accurately explained through species reproductive behaviors and strategies of both adult and juvenile birds (Desrochers and Hannon, 1997). In particular, many species recorded at forest sites were observed to be undertaking a number of breeding activities, including courtship and territorial displays, nest construction and food gathering during spring. Subsequently, sightings of juvenile birds at forest and road sites became more frequent during summer (personal observation). Importantly, the timing of these activities and movements, particularly those of the gray fantail and noisy miner, are consistent with observations previously reported by Griffioen and Clarke (2002) and Ford et al. (2000).

CONCLUSIONS AND IMPLICATIONS

This study, to the knowledge of the authors, is the first in Australia to have investigated, assessed and evaluated the impact of roads on bird richness and crossings. Its key findings were that both forest species richness and numbers of road crossing species declined significantly with increasing road width and that these negative effects were most obvious for the small forest dependent guild compared to large forest dependent, honeyeater and urban tolerant guilds. Moreover, numbers of road crossing individuals also declined significantly with road gap width and were most obvious in small birds (<20 cm). Finally, species compositional similarities at both forest and road sites increased slightly between spring and summer, suggesting some seasonal variation to be present within the data. These findings are of value to the understanding of both conservation and the ecological role of birds in ecosystem health and functioning through the provision of a number of beneficial services, such as pollination, seed dispersal and arthropod control.

Therefore, it is strongly recommended that wildlife movement measures be implemented to ensure connectivity between forest fragments within Brisbane. Specifically, such solutions should be tailored toward improving gap permeability along individual roads. For example, vegetation structure and complexity alongside smaller roads should be maintained and enhanced where necessary through the provision of a dense understory to promote richness crossing of small forest dependent species while simultaneously reducing competition and predation from larger birds (Desrochers and Hannon, 1997; Ford et al., 2000; Kutt and Woinarski, 2007; Tremblay and St Clair, 2009; Jones and Bond, 2010; Jones et al., 2011; Thinh et al., 2012). However, it should be noted that encouraging species to cross roads may in turn expose them to greater risk of mortality, the implications of which become far greater in instances when rare species are involved (Jaeger and Fahrig, 2004). Alternatively, serious consideration should be given to the implementation of vegetated wildlife overpasses over larger roads and motorways. Although costly, this method has previously proven to be highly successful in promoting the movements of several species and has additional mitigation value for multiple taxa beyond birds (Jones and Bond, 2010; Jones et al., 2011; Jones, 2014; Pell and Jones, 2015). Thus, the cost may be high and perhaps unjustifiable for birds alone, but where vegetated overpasses are built for multiple species, benefits can accrue for birds if design objectives include bird passage.

Further studies should also be undertaken of the impacts of gaps in vegetation continuity not associated with vehicular activity (i.e., forest tracks, park walkways) on bird movements, the relationship between traffic volume and species risk-avoidance strategies, and the impacts of roads on the genetic and ecosystem levels, so as to better inform future planning and species conservation. This is especially important in the face of continued urbanization contributing to rapid habitat fragmentation and species displacement around the world.

AUTHOR CONTRIBUTIONS

Conceived the experiments: CJ, DE, and DJ. Performed the experiments: CJ. Analysed the data: CJ. Contributed materials/critique/analysis tools: CJ, DE, and DJ. Wrote the paper: CJ.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2017.00036/full#supplementary-material

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On-Board Video Recording Unravels Bird Behavior and Mortality Produced by High-Speed Trains

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Large high-speed railway (HSR) networks are planned for the near future to accomplish increased transport demand with low energy consumption. However, high-speed trains produce unknown avian mortality due to birds using the railway and being unable to avoid approaching trains. Safety and logistic difficulties have precluded until now mortality estimation in railways through carcass removal, but information technologies can overcome such problems. We present the results obtained with an experimental on-board system to record bird-train collisions composed by a frontal recording camera, a GPS navigation system and a data storage unit. An observer standing in the cabin behind the driver controlled the system and filled out a form with data of collisions and bird observations in front of the train. Photographs of the train front taken before and after each journey were used to improve the record of killed birds. Trains running the 321.7 km line between Madrid and Albacete (Spain) at speeds up to 250–300 km/h were equipped with the system during 66 journeys along a year, totaling approximately 14,700 km of effective recording. The review of videos produced 1,090 bird observations, 29.4% of them corresponding to birds crossing the infrastructure under the catenary and thus facing collision risk. Recordings also showed that 37.7% bird crossings were of animals resting on some element of the infrastructure moments before the train arrival, and that the flight initiation distance of birds (mean ± SD) was between 60 ± 33 m (passerines) and 136 ± 49 m (raptors). Mortality in the railway was estimated to be 60.5 birds/km year on a line section with 53 runs per day and 26.1 birds/km year in a section with 25 runs per day. Our results are the first published estimation of bird mortality in a HSR and show the potential of information technologies to yield useful data for monitoring the impact of trains on birds via on-board recording systems. Moreover, recordings point to the use of the infrastructure by birds as a key issue leading to bird train-kill.

Keywords: agrarian birds, environmental impact assessment, flight initiation distance, high-speed railway, infrastructure, monitoring, camera, road kill

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INTRODUCTION

Railway has substantial growth potential as a mode of transportation given its energy efficiency, low emissions, and low perceived environmental impact relative to the other alternatives (Profillidis et al., 2014; Dorsey et al., 2015). Economic development is closely linked to increased mobility of people and goods, which can drive continued global growth of passengers, goods volumes, and length of transportation infrastructure (Chapman, 2007; Dritsaki and Dritsaki, 2014). However, to address environmental effects, especially energy consumption and greenhouse gas emissions, economic development strategies must define transportation plans that maximize benefits and minimize environmental costs (EC, 2011; Dulac, 2013; Liu et al., 2013). Railroad transportation is thus becoming an outstanding component of such strategies (Dulac, 2013; UIC, 2016).

High-speed railways (HSRs) are key to the development of high-capacity transportation networks that can compete successfully with air transport for medium distances. For instance, the European Union is planning to double the size of its HSR network by 2030, aiming for the majority of passenger travel to occur by rail by that time (EC, 2011). For routes in which high-speed trains compete directly with air travel, such as, between Madrid and Barcelona, Spain (624 km), once trains are operational, they partially absorb passengers from other modes of transportation (Castillo-Manzano et al., 2015). Medium term, supply and demand patterns for air travel and HSRs adapt to the new market conditions (Pellegrini and Rodriguez, 2013) and the disadvantages of each mode of transportation are reduced if demand is stable (Albalate et al., 2015; D'Alfonso et al., 2015). In this context construction of HSR networks is planned in different parts of the world as a priority mode of intercity connection (Campos and de Rus, 2009; USDOT, 2009; Todorovich et al., 2011; Fu et al., 2012).

However, HSR planning faces a key environmental challenge: practically nothing is known regarding the potential environmental effects of HSRs beyond pollution and climate change (Popp and Boyle, 2017). The destruction of habitats and their degradation close to the infrastructure (edge effect) is thought to be less important for railroads than for highways given the smaller breadth of its infrastructure and lower traffic intensity (Dorsey et al., 2015; Borda-de-Água et al., 2017). It has also been suggested that vertebrate mortality from collisions should not be a major problem because of the relatively low frequency of train circulation, although under certain conditions, it can become a relevant issue and perhaps a bigger problem than for roads running parallel to the railways (Waller and Servheen, 2005). In the case of birds, collision frequency with high speed trains is practically unknown (e.g., Loss et al., 2015), but could be high since (i) birds use areas surrounding railways and can even be attracted to them (e.g., for traditional rail lines Havlin, 1987; De la Pe-a and Llama, 1997; Mammen et al., 2002; Li et al., 2010; Wiaçek et al., 2015), (ii) slow flight birds are less able to avoid rapid trains, being then more often at risk of road or railroad-kill (Glue, 1971), and (iii) trains run at such high speeds that birds cannot evade an oncoming train (DeVault et al., 2015).

Because it's difficult to work within and around HSR networks, scientific knowledge of bird mortality from collisions is very scarce (see reviews by Dorsey et al., 2015 and Santos et al., 2017). The infrastructure is fenced and highly restricted to human access for safety reasons, making it very difficult to run field studies (Wells et al., 1999). Also, bird carcasses from train collisions are left in a condition which makes location and identification difficult, especially in the presence of scavenger animals around railways (Heske, 2015; Reyes et al., 2016). Besides, the sheer breadth of rail networks and cost of fieldwork make it practically impossible to conduct studies that cover even a small part of a network. Such work is thus feasible only along short stretches of the line. The preliminary results of an ongoing field study along 10.6 km of HSR in Spain (LIFE+ Impacto Cero, 2017) show bird mortality of about 91.3 birds/km/year, with species being affected in accordance to their abundance (Malo et al., 2016).

Information technologies may be critical to overcome the difficulties inherent in estimating HSR bird mortality, specifically the use of on-board systems that record collisions. Such systems should include (i) a cabin-front recording camera, (ii) image and data storage of the instant the events occur (location, speed, etc.), and (iii) data review and interpretation to enable extraction of only relevant data from the huge volume of data collected. Technology advances have already addressed the first two requirements and the emergence of an artificial intelligence system may soon enable automated analysis of the generated images (Yu et al., 2013; Longmore et al., 2017).

While awaiting technology advances, there is, thus far, no experience with applying extant technology to the problem of HSR bird mortality, although some illuminating work has been done in car-dragonfly collisions (Furness, 2014). For HSRs, on-board systems could solve safety, cost, and spatial extent issues that today preclude even a basic estimation of bird mortality.

The objectives of this article are (i) to demonstrate the use of a high-speed image recording system aboard high-speed trains for analyzing behavior and mortality of colliding birds, and to discuss the potential uses of such a system; and (ii) to present initial estimates of bird collision mortality and associated data on bird behavior during train approaches, which will be valuable for developing corrective measures.

MATERIALS AND METHODS

We recorded bird collisions with trains using a portable system comprising a high-speed digital camera and a data logger with high-recording-frequency GPS navigation (**Figure 1**). The video camera was a commercial model (GoPro Hero 3+ Black Edition) that records in a super-wide format (170°) at 120 frames per second (FPS) and 1,280 × 720 pixel image resolution or 240 FPS with 848 × 480 pixel image resolution. After a few initial recordings at 240 FPS, we decided to use 120 FPS to achieve clearer images of the collision events. The small camera size enabled us to anchor it with a suction cup to the front windshield of the train without loss of visibility for the train driver. For recording and precise geolocation of the images, we used a professional data logger with GPS (Qstarz

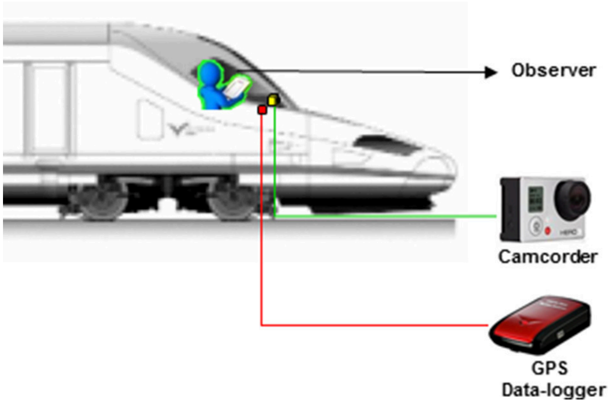

FIGURE 1 | Basic diagram of the collision detection system for a high-speed train. In the train cabin, a high-speed camera was aimed forward, connected to a data logger that records GPS navigation data continuously. In parallel, an observer records on a form the crossing and collision of birds with the train.

BT-Q1000eX) with a 10-Hz location-refreshing frequency. GPS data were processed with the QRacing^{TM} v3.3.3 software (Qstarz International Company Ltd.) and they were synchronized with data from the camera with Dashware$^{©}$ (GoPro Inc.). We installed the system in the front cabin of high-speed trains (GEC Alstom-series S100-and Talgo-Bombardier-series S112 and S130) from Renfe Viajeros in regular circulation on the line running at speeds up to 250–300 km/h.

In parallel with video recordings, an observer standing in the cabin behind the driver filled out a form to note trip data and observation of birds in front of the train (time, location, bird size, number of individuals, and behavior) as well as collisions detected from the cabin. We used these data to focus the review of each video on the points of the line where birds had been detected (see below). Also, for each trip, we photographed the front of the train before and after the trip, to study collision evidences (feather remains and blood) that are often found on the train bodywork in search of additional collisions not seen from the cabin.

All the filming was done between Madrid and Albacete stations of the high-speed line, 321.7 km apart (Supplementary Figure 1). The rail route crosses mostly rural areas with dryland crops (∼60%; south of Madrid, Mesa de Ocaña-Tarancón, La Manchuela) and irrigated land (∼10% near Albacete), as well as forest and scrubland areas (∼20% around Cuenca). The line also crosses peri-urban and industrial zones (∼8%) and river meadows (∼2%), especially near the Tajo and Júcar river crossings. Part of the line passes near two Special Protection Areas of ornithological interest linked to steppe and rural habitats (Natura 2000 site ES0000170) and marshlands (Natura 2000 site ES0000119). The line also crosses several protected areas of pseudo-steppe habitat interest.

Most of the railway runs on the land surface and the design of the rail line permits speeds above 250 km/h. Because the slopes do not exceed a 2‰ (0.2%) grade, the rail line runs at the level of the land in the flattest parts, and on embankments, mostly 2- to 15-m high, where the terrain is uneven. The line includes 20 tunnels between 187 m and 3,128 m long, 7 of which are more than 1-km long. There are also 30 viaducts between 127 m and 1,600 m long. The HSR line runs 30 trains per day on working weekdays and 11 trains per day on holidays, with a journey duration between Madrid and Albacete of 1 h 30 min (average speed 214 km/h). On the same line, trains run between Madrid and Motilla del Palancar (km 251) toward Valencia with frequencies of 30 runs on work days and about 25 on holidays. Trains regularly run during daytime, though some circulations take place at nighttime short before dawn o after dusk, mainly in winter.

We conducted the recordings between July 2014 and May 2015, with a total of 66 recording journeys at daytime distributed across seasons (14–20 recordings per season). Two journeys were done every filming day, one early in the morning beginning around dawn from Madrid and the return trip from Albacete in the late evening. Our sampling was thus balanced between mornings and evenings, though logistic restrictions precluded a fully balanced sampling along the whole day. The system could not operate at nighttime with the front lights of the train, thus leaving a full gap of knowledge for this situation. All recordings (total recording time of 59 h and 55 min) were downloaded to a hard drive for analysis. The recordings covered about 14,700 km of cumulative travel (average 222.7 km per recording, range 78.3–288.7 km).

A single investigator carried out film data extraction by direct viewing each recording at all the points where the cabin observer had indicated the presence of birds (about 500–1,000 m in each case). In addition, in all the recordings, we reviewed the video for two stretches of the line at km 83–89 and 96–101 (total, 10.6 km), where there are ongoing field studies of bird behavior and mortality (Malo et al., 2016, 2017). This additional review, along with some review of other small portions of recordings, comprised about 5% of the total recording time.

From each bird observation, we extracted the following data: number of individuals, taxonomic identification as precise as possible, position (resting/flying) at the first frame in which the birds were visible, time, location (geographic, and kilometer position), train speed, surrounding habitat, typology of the rail line, and if birds flew across the line. For resting birds we noted if they were using elements of the infrastructure (wires, poles...) or not. In the case of crossings, we differentiated between those that occurred above the catenary or between the rails and the catenary located at a 5.3-m height. The gap between the rails and the catenary defines the bird-train collision risk area.

In recordings where the birds were at rest at first detection, we reviewed the image sequences to estimate the birds' distance from the train when they started their escape flight (FID, flight initiation distance). FID is a common measure of the bird response to human disturbance or predation risk (Weston et al., 2012), and it is directly related with the remaining time to escape from the approaching train. We used the poles supporting the catenary, which are 60 m apart, as a distance reference. Also, for each sequence, we determined whether or not the observation resulted in a bird collision with the train.

To estimate the mortality rate due to collision (colliding birds per km traveled), we counted the number of colliding birds directly recorded by the camera on each trip and added the number of collision marks on the front of the train that did not correspond to observed collisions. We used this estimate of mortality per km traveled to calculate the annual bird mortality for the two line sections, Madrid-Motilla del Palancar with about 53 runs per day and Motilla del Palancar and Albacete with 25 runs per day.

Temporal patterns of bird observation from the cabin were analyzed through a chi-squared test comparing the number of observations in morning vs. afternoon journeys. Temporal patterns were not further analyzed due to the time and spatial restrictions of our recordings (uneven in time and beginning every morning from Madrid). To better understand the underlying factors leading to mortality, FID variability among bird groups was analyzed via one-way ANOVA. For this analysis we considered only observations from bird groups with more than 10 records in order to work with more reliable estimates of this variable. Four bird groups were thus considered according to taxonomical and ecological criteria (passerines, pigeons and doves, corvids, and mid-sized raptors). Also, a t-test was carried out to compare the speed of the train in events of bird crossing under the catenary ending in collision vs. those in which the bird escaped. All analyses were carried out with STATISTICA 8.0 (StatSoft, Inc., 2007).

RESULTS

For 66 journeys, we obtained a total of 1,090 recorded observations of birds from the cabin (**Figure 2**) distributed throughout the line (Supplementary Figure 1). Of these, 1,040 were seen directly by the observer and 50 (4.6%) were obtained during film review. Considering all of the raw data, we estimated an average frequency of 16.5 observations per journey (range 0–41) and 0.07 observations per km traveled (i.e., one observation every 13.9 km). Observations were more frequent in the morning journeys than in the afternoon ones (65.0 vs. 35.0%; chi-squared test = 48.8; d.f. = 1; $p < 0.001$), and they probably peak with the first lights of the morning.

The recording quality enabled at least a partial taxonomic identification for most of the observations (Supplementary Table 1), at a species level in 45.5% of the cases and at a supra-species level in 52.0% of the cases. In 1.5% of the observations, we could not determine the type of bird. We identified a total of 32 different bird species of which 62.5% were non-passerines (especially daytime raptors, *Falconiformes*, including eight separate species) and the remaining 37.5% were passerines (especially corvids, *Corvidae*, including four species). Based on the number of observations, passerines predominated with 52.2% of recordings (30.5% corvids and 21.7% other passerines), followed by 27.6% of pigeons and doves (*Columbiformes*, especially the common wood pigeon, *Columba palumbus*). We also detected daytime raptors (*Falconiformes*; 16.4%) and other non-passerine species of various orders (2.3%; Supplementary Table 1).

In terms of flock size, most bird observations were of singletons (70.1%) and bird pairs (13.4%). We recorded flocks of 3–10 individuals in 12.8% of the cases and flocks of 11 to 50 individuals in 2.7% of the cases. Only in 1% of the cases did we observe more than 50 individuals (including a flock of spotless starlings, *Sturnus unicolor*, of ~350 individuals).

In terms of infrastructure crossing behavior, in most of the observations (80.5%), birds crossed the infrastructure, with 51.0% flying over the catenary and 29.4% flying under the catenary in the collision risk zone. The birds that did not cross the rail line mostly stood on the infrastructure (e.g., on power

FIGURE 2 | Image capture of a recording from a train running at high speed. Lower left quadrant, in white, shows data inserted by the GPS system. Red arrow shows bird position moments before collision with the train.

lines or poles) or escaped from the infrastructure with no risk of collision. Only 2.3% of the observations were of birds that did not cross the infrastructure nor used it for resting. A relevant feature is that 27.1% of the line crossings were of birds initially resting on some element of the infrastructure (rails, catenary, fencing, etc.); this portion reached 37.7% for birds crossing within the collision risk zone.

In 162 cases, we could determine the FID and found significant differences [ANOVA $F_{(3,\,158)} = 31.55$, $p < 0.001$] between different groups of birds (**Table 1**). Thus, there was a gradient in which the birds with lower FIDs were passerines (7 *Galerida cristata*, 1 *Lanius meridionalis*, and 4 indeterminate), followed by pigeons and doves (79 *C. palumbus*, 8 *Columbia livia*, and 2 *Columba* sp.), corvids (10 *Corvus* sp., 3 *Corvus corone*, 1 *Corvus corax*, 2 *Corvus modenula*, and 6 *Pica pica*), and finally midsize and large raptors (18 *Buteo buteo*, 1 *Milvus milvus*, 1 *Milvus* sp., 2 *Aquila chrysaetos*, and 18 indeterminate). In 11.2% of the recordings of pigeons and doves, the birds did not take flight as the train passed (i.e., FID = 0), but rather remained at rest on the catenary cables.

Finally, we counted a total of 42 bird collisions, 28 of them recorded from the cabin. Collisions were recorded in 47.0% of the journeys. As a whole, the data are equivalent to a mortality of 0.0029 birds per km traveled by a high-speed train (i.e., one collision every 349.8 km). With respect to average traffic on the HSR in this study, the mortality is estimated to be 60.5 birds/km year on the Madrid-Motilla del Palancar section and 26.1 birds/km year between Motilla del Palancar and Albacete.

For colliding species, among 28 cases recorded from the cabin, 53.6% were small passerines, 14.3% were pigeons and doves, 10.7% were corvids, and 10.7% were other non-passerines (**Table 2**). In 10.7% of the cases, we could not determine the species or group of the colliding bird. The percentage of observations from each species ending in collision was very variable (**Table 2**), though these features reflect a mix of both interspecific differences in flight behavior and size-related differences in detectability (see also Supplementary Table 1).

In 12.1% of the observations of birds crossing under the catenary there was a collision. All collisions occurred in high-speed situations (mean ± standard deviation, 265.8 ± 39.2 km/h; range, 175–305 km/h; $N = 20$), with no significant differences (t-test = 1.06; d.f. = 201; $p = 0.291$) relative to the remainder of line crossings under the catenary (251.5 ± 58.9 km/h; range, 0–305 km/h; $N = 183$). Notably, the recordings showed that 17.8% of the birds that collided were individuals initially resting on an element of the infrastructure when they were first visible in the recording.

TABLE 1 | Flight initiation distance (mean ± SD) of birds facing an approaching high speed train.

Bird group	No. observations	Flight initiation distance (m)
Passerines	12	59.6 ± 33.5
Pigeons and doves	89	67.8 ± 36.8
Corvids	21	106.2 ± 42.2
Mid-sized raptors	40	136.0 ± 49.1

Data correspond to bird taxa with a sample size larger than 10 observations.

DISCUSSION

The present results show the potential of information technology in analyzing the impact of high-speed trains on bird communities in the areas they cross. In addition, the data presented provide a first approximation to direct bird mortality from high-speed trains, and enable an initial understanding of some key aspects of bird behavior that lead to mortality risk.

It is important to note that the estimation of collision mortality seems reliable in the light of the available evidences (see below), even though it was obtained with a simple and portable system. The estimate of bird collisions obtained for the Madrid-Motilla del Palancar section (60.5 birds/km/year) was of the same order of magnitude as that measured in a simultaneous sampling over an entire year on two stretches totaling 10.6 km (91.3 birds/km/year; Malo et al., 2016). The difference could be due to the spatial variability in abundance of birds surrounding the rail lines (Supplementary Table 1) and the fact that field studies were carried out in an area adjacent to a Special Protection Area of ornithological interest (Natura 2000 site ES0000170). Moreover, the lack of a balanced sampling along the whole day and the absence of nighttime recordings add some uncertainties of unknown direction to the estimate. It is noticeable in this sense that owls are known to die in traditional railways (Glue, 1971; De la Pe-a and Llama, 1997), and HSR have some nighttime circulations that also lead to owl death (Malo et al., 2016). Such mortality associated to trains running at night could not be analyzed here, and it probably affects few but completely different species as those recorded here. In conclusion, further recordings including nighttime would be needed for a detailed assessment of bird mortality in HSR, but our data can be seen as a first reliable approach to it. In the scientific literature, there are only

TABLE 2 | Number of bird collisions by species recorded from the train cabin, and percentage of recordings ending in collision with the train.

Bird group	Collisions	Percentage of recordings
PIGEONS AND DOVES		
Columba palumbus	3	1.6
Columba livia	1	3.1
OTHER NON-PASSERINES		
Upupa epops	1	25.0
Apus apus	1	100.0
Tachymarptis melba	1	100.0
CORVIDS		
Pica pica	2	1.4
Corvus sp.	1	0.6
SMALL PASSERINES		
Sturnus unicolor	2	6.9
Galerida cristata	1	12.5
Motacilla alba	1	100.0
Emberiza calandra	1	100.0
Chloris chloris	1	100.0
Delichon urbica	1	100.0
Undetermined passerine	8	4.2
Undetermined	3	18.8

internal rail company documents recording bird mortality for high-speed trains, and published studies for traditional lines to date are based on very targeted field carcass samplings (Havlin, 1987; De la Pe-a and Llama, 1997; Mammen et al., 2002; Heske, 2015). The lack of data on the impact of trains on fauna extends to all types of rail lines and compromises their environmental evaluation (Wells et al., 1999; Rodríguez et al., 2008; Dorsey et al., 2015).

This is the first time that a system such as, ours has been used to study bird collisions with trains, although a similar system was used to study dragonfly-car collisions (Furness, 2014). To date, few studies have used data obtained in train cabins from direct observation (Wells et al., 1999) or driver surveys (SCV, 1996). Video recording observations combined with collection of GPS data improves the collection of precise data, both for identifying the species involved and for determining location and circumstances of the collisions (train speed, bird behavior, etc.). Techniques like video recording from fixed points, vehicles, or animal-borne cameras have been applied to study animal behavior and monitor wildlife (Whorff and Griffing, 1992; Thompson et al., 1999; Okuyama et al., 2015), but rarely to assess mortality risk (Desholm et al., 2006; Cryan et al., 2014; Furness, 2014; Doppler et al., 2015).

As for the types of birds crossing the lines and colliding with trains, our results showed the presence of birds common to the agrarian landscapes along the railway, and especially those birds that use the infrastructure as a habitat for feeding, resting, and even nesting (Havlin, 1987; Li et al., 2010; Morelli et al., 2014; Malo et al., 2017). Most observations were done early in the morning and corresponded to passerines, many of which could not be identified precisely. We recorded pigeons and doves, large corvids (most of them probably *C. corone*), and midsize corvids (e.g., *P. pica*), frequently. But we also observed raptors such as, *B. buteo* in several occasions as well as other species that might approach the railway looking for carcasses or prey, primarily rabbits and rodents (see for motorways Planillo et al., 2015). On the whole, the collision data are relatively worrisome from a biological perspective, involving a large number of individuals, some portion of which may be protected species (Mammen et al., 2002; see also Malo et al., 2016). From the point of view of train operation and safety, the possibility of collision with large birds or with large flocks is not insignificant, given the potential cost of stopping the trains and repairing the damage (Seiler et al., 2014; Renfe Viajeros, unpublished data).

Our mortality estimates via on-board camera systems are also useful for quantifying the time and cost savings achievable with the use of information technologies. To obtain cabin data, we invested the recording time (an observer for 33 days of round trips) and the time for review and assembly of the data (an ornithologist working for about 50 days). In comparison, field data collection in the two sub-stretches of rail line required a group of at least three ornithologists and one safety technician during 48 days (4 seasons * 6 surveys * 2 sub-stretches) as well as parallel installation of baited stations to estimate carcass removal rates and carcass detectability (Reyes et al., 2016). Added to these resources, would be preparation time for field material and datasets (total of around 24 person-days). In short, on-board recording systems can enable much more efficient data collection than traditional methods based on direct personal observation (83 vs. 216 person-days in this example), underscoring the current trend of increasing reliance on information technology for ecological studies (Tomkiewicz et al., 2010; Galliard et al., 2012; Gurarie et al., 2016; Price and Schmitz, 2016). In our case, automation not only saved labor costs but also enabled us to broaden the study beyond what is possible with direct fieldwork to the more than 300 km that comprise the entire rail line.

In addition, the on-board recordings enabled us to describe three factors of bird behavior that determine mortality. Thus, (i) we confirmed that a large percentage of observed birds cross the rail lines under the catenary and are thus at risk for collision. Slightly more than one of every three recorded crossings occurred in the risk zone, demonstrating that birds do not perceive the risk of crossing the gap between the rails and a power wire located at a 5.3-m height. Although this estimate may be subject to some bias, given that on-board recordings may underestimate events above the catenary, the results are informative, showing a high of collision risk during low flight, a routine characteristic in bird flight patterns (Shamoun-Baranes et al., 2006; Wulff et al., 2016). Also, (ii) we confirmed that the use of various infrastructure elements by birds (poles, catenary, embankments) strongly determines their risk of collision (Morelli et al., 2014; Mainwaring, 2015); almost 40% of the crossing events under the catenary occurred for birds resting on the infrastructure moments before the train arrival.

Lastly, (iii) the recordings showed that, frequently, the birds reacted to the train's approach when it was almost upon them. The escape distances for different groups of birds (60–140 m) correspond to flight initiation when the train traveling at 300 km/h is only 0.72 to 1.68 s away from the resting point. The data thus demonstrate the problem faced by many birds: too late response to avoid collision with the high-speed train. This is probably the general rule, even though recordings do not show average behavior of all birds since the on-board system only registers events relatively close to the train. Thus, it misses birds that react early and escape upon commencement of ground and catenary vibration, which occurs 5–10 s before train arrival (personal observation). The delayed reaction is in accordance with the limited capacity of the birds to react to objects approaching at high speed (Martin, 2011; DeVault et al., 2015), and depicts the reduced probability of wildlife to escape from an approaching high-speed train.

On technical grounds, the system used for this experiment is relatively low cost and available on the market. However, there are other technologies and equipment, which are more expensive and less accessible, that have enormous potential to advance this field of work. Our GPS navigation system often lost signal due to tunnels or cabin interference, but future systems could use the automated position controls present in the train itself. The recordings, which were carried out mostly at 120 FPS and

1,280 × 720 pixel resolution, enabled us to identify bird species in about 45% of the cases. The identification most often was only possible after a detailed review of the complete recording sequences and was based as much on details of the birds in the images with sufficient definition as on the flight patterns observed in the sequence as a whole. Modern cameras can record up to 4.4 trillion FPS (Nakagawa et al., 2014) and 7,580 × 4,320 pixel cameras also exist on the market (e.g., WEAPON 8K S35) for very specific applications, but they have limited recording capacity in terms of recording time due to the data volume generated. In this trade-off between recording speed and image quality, it is probable that, in the near future, cameras capable of 480 FPS and 4,096 × 2,160 pixels may be available at affordable prices for applications such as, our study. With such images, it might be possible to recognize at a species level most of the birds that cross in front of the train, especially in cases where images show them very close to the train, a brief instant before collision.

Additionally, artificial intelligence systems under development are expected to enable extraction of useful wildlife data from images taken in nature by automated cameras (e.g., Yu et al., 2013; Longmore et al., 2017). This extraction capacity will be a key advance to enable data analysis of high-speed recordings from train fronts, similar to the extraction of useful biological monitoring data from continuous recording webcams or sensor networks (Benson et al., 2010; Porter et al., 2010; Evans et al., 2015). In our case, review of the recordings was focused on the points at which the cabin observer detected birds in front of the train, and on a small complementary sampling of the recordings as a whole. Since review of the image sequences must be done at a slow speed (24 FPS) or even frame-to-frame, the time spent for reviewing is much longer than the length of the sequences of interest and it limits a generalized use on the trains (see also Longmore et al., 2017). In any case, automating data interpretation of the on-board recordings will be a challenge. The rails, posts, and catenary that comprise the infrastructure limit the analysis focus, but the constant (relative) movement of the background makes it difficult to distinguish the targets (birds) from the landscape elements in the recordings (Yu et al., 2013; Longmore et al., 2017). A solution for this would be to combine high-speed cameras with complementary sensors (e.g., sound/impact) in the front of the train, to precisely record collision impacts, and in combination with automated recording systems capture specifically the video fragments for the moments just before a collision occurs. Such a solution would also limit the volume of information to be stored in hardware. Future potential for improvement of the system is thus huge.

Anyhow, the system in our study shows the present and near-future potential for such high-speed recording systems boarded on trains to assess the environmental impact of HSRs. First, information gleaned from massive data on bird crossings and collisions with trains in different settings (landscapes, seasons, time of the day) would be of high interest for proper environmental assessment of new and planned rail infrastructure as a viable travel option in the future (USDOT, 2009; EC, 2011; UIC, 2015). Collision mortality data may command attention in railways in the same way it already does in roads (Benítez-López et al., 2010; Dorsey et al., 2015). Second, high-definition recordings will provide a better understanding of how, where, and why collisions occur, opening the door for corrective measures (Rodríguez et al., 2008; Gunson et al., 2011). Longer term, these onboard systems may also be able to feed data to the Environmental Monitoring Programs of these rail infrastructures, continuing to improve both prevention and mitigation (Williams and Brown, 2014). At present, our data imply that (i) mitigation should be focused on reducing the proportion of birds flying under the catenary for instance by use of barrier-like structures, at least in locations of high collision risk (see Rodríguez et al., 2008; LIFE+ Impacto Cero, 2017). Also, (ii) efforts should be aimed at reducing the attractiveness of rail surroundings to birds, not only structural elements (poles, catenary), but also the habitat of the embankments (Malo et al., 2017), as well as reducing the potential of dense populations of prey in embankments that can attract birds (Planillo et al., 2015). Unfortunately, addressing the small escape distance for the birds may not be possible given the sensory and behavioral limitations of birds (Martin, 2011; DeVault et al., 2015).

AUTHOR CONTRIBUTIONS

EG, JM, IH, CM, SG, and JH contributed substantially to different parts of the study design, data acquisition, analysis and interpretation. First text was drafted by EG and JM with contributions from IH, CM, SG, and JH, and final paper was revised and approved by EG, JM, IH, CM, SG, and JH. EG, JM, IH, CM, SG, and JH are accountable for all aspects of the work regarding their accuracy and integrity.

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SUPPLEMENTARY MATERIAL

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Roadside Abundance of Anurans within a Community Correlates with Reproductive Investment

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Roads and their associated effects (road-kill, pollution, etc.) have a largely negative impact on animals, especially amphibians, but not all species are affected to the same degree. Variation in life histories may explain some of these differences. Here, we examine how abundance of anuran species in roadside habitats is correlated with an aspect of reproductive life history: number of eggs produced by a female per year. Using data from a 1.5-year monitoring project in Central Florida, we found a positive correlation between the number of eggs produced by an average female of a species and the proportion of individuals found in roadside habitats compared to control habitats. This implies either that populations of species with a greater reproductive rate are able to rebound more quickly from negative road impacts, or that there is a strong selective pressure on species with low reproductive rates to avoid roads.

Keywords: amphibian, conservation biology, frog, road ecology, transportation

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INTRODUCTION

The field of road ecology still seeks answers to fundamental questions, including which species are most at risk when roads are created and why. In most cases, roads have a negative effect on animal populations (reviewed in Fahrig and Rytwinski, 2009). However, some species are more negatively impacted than others. One of the most common effects of roads is reduced abundance. A recent meta-analysis found that the magnitude of this effect varies with life history traits; in particular, roadside abundance decreases as reproductive rate decreases (Rytwinski and Fahrig, 2012). In essence, species with low reproductive rates and late ages at maturity are more vulnerable than species which breed prolifically and mature quickly.

Anurans (frogs and toads) are currently experiencing severe declines worldwide, with local and species-level extinctions (Alford and Richards, 1999; Beebee and Griffiths, 2005; Halliday, 2005). Unfortunately, they also appear to be one of the taxa most negatively affected by roads (reviewed in Andrews et al., 2008; Fahrig and Rytwinski, 2009). In addition, the meta-analysis by Rytwinski and Fahrig (2012) found that anurans exhibit the aforementioned relationship between life history traits and abundance near roads, where species with a low reproductive rate are less abundant in roadside areas.

While meta-analyses are powerful, they present a general trend for the group being considered, often using species from distant areas of the world, and may not provide insight on a fine scale, i.e., the local or community level. Factors like interspecific competition and/or differences in habitat preference (Creusere and Whitford, 1976; Sazima and Eterovick, 2000) may have a greater influence than life history traits on the pattern of anuran abundance near roads within a community. However, if the relationship between life history and roadside abundance does hold on a smaller scale, roads could have a strong influence on community composition. Rytwinski and Fahrig (2011) investigated the relationship between mammal body size, mobility, and reproductive rate and traffic density within a landscape and found that reproductive rate is an explanatory factor in roadside community structure, echoing the global trend seen in the meta-analysis. Here, we investigate whether the community-level correlation between reproductive rate and roadside abundance also holds for anurans.

We assessed the relationship between reproductive rate and roadside abundance in anurans in Ocala National Forest (Florida, United States). Amphibians in general appear to be highly susceptible to traffic mortality and anurans have been identified as highly vulnerable to road effects (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2012; Smith, 2012; Beebee, 2013). We predicted that populations of species that produce larger numbers of offspring would be better able to compensate for these negative effects and would be found in higher abundances near roads.

MATERIALS AND METHODS

Trap Surveys

We examined data collected along two sections (≈3.5 km each) of State Road 40 (Ocala, Florida, United States) from February 2012–July 2013 as part of a study for the Florida Department of Transportation (FDOT) to assess animal species richness and abundance (including anuran abundance) to collect baseline data prior to expansion of the highway (**Figure 1**). Monitoring the area for at least a year allowed us to observe most anuran species in the area, whose activity levels vary throughout the year by species (e.g., winter breeders vs. summer breeders). Because the goal of data collection was to thoroughly sample the different habitat types along the highway, our extensive monitoring of this area generated a large biological dataset. The stretch of SR 40 that we studied is located in Ocala National Forest and Silver River State Park, in Marion County, and has an annual average traffic volume of about 11,000 vehicles/day. All other roads within 5 km of the study sites were either unpaved trails or had traffic volumes <2,000 vehicles/day (**Figure 1**). Such low traffic volumes have been shown to have little to no effect on anuran abundance (Eigenbrod et al., 2008a). The primary vegetation types in the area are wet pine flatwoods, hardwood swamp, and upland mixed hardwood-pine forest.

Within the two study sections, we subsampled the area with roadside and control traps to monitor anuran abundance in the area. Each roadside trap consisted of a 30-m long drift fence positioned parallel to the road in the right-of-way (∼10–20 m from the road surface). Four bucket traps and four screen funnel traps were positioned along each drift fence. Control traps followed the same design, but were placed ∼500 m from the road. Due to heterogeneity of environmental conditions (vegetation, water table, etc.) it was impossible to space control traps at completely uniform distances from the road (mean 558 m; range 315–870 m). To ensure that our subsampling effort was uniform between roadside and control areas, we were careful to place roadside and control traps equally within the different vegetation types found in the area (wet pine flatwoods, hardwood swamp, and upland mixed hardwood-pine forest). These three habitat types are important to the anuran species in the area during different times of the year (ephemeral pools in flatwoods for egg deposition, permanent water in swamps for aquatic species, and upland forest during the non-breeding season for terrestrial species) so sampling in all three increased our coverage of the area and the likelihood that we would trap the majority of anuran species.

Traps were checked in the morning for 3–4 consecutive days each week. During periods when traps were not being checked regularly, they were shut to prevent animals from entering. All animals found in the trap arrays were identified to species and age class and marked in order to note recaptures.

This research was approved by the Institutional Animal Care and Use Committee at the University of Central Florida under protocol #11-37W and conducted under Scientific Collecting Permit #LSSC-11-00092.

Measurement of Reproductive Investment

We used the average number of eggs/yr produced by a female (eggs/clutch*number of clutches per year) as a measure of the reproductive investment of each anuran species. To avoid autocorrelation, we chose not to use age to reproductive maturity of females as an independent variable because age at maturity correlates with body size, which greatly influences the number of eggs produced (Gibbons and McCarthy, 1986; Berven, 1988).

To determine the average number of eggs laid by females of each species in a year, we referenced two guides to Florida amphibians (Ashton and Ashton, 1988; Bartlett and Bartlett, 2011). If the number of eggs was reported as a range (for example, 200–300 eggs), we took the average of the two values (e.g., 250 eggs). If it was reported as being "up to" a certain number, we halved that number, as it is likely that these reports were of extreme observations. If it was reported as "more than" a number, we reported that number. Obviously, this method of determining reproductive rate is by no means exact, but we believe that it is successful in approximating the true pattern (i.e., "this species generally produces more eggs, while this species generally produces fewer"). We then used the average from the two guides as the final number. If only one book gave information for a particular species, we used the value it reported. If no information for that particular species or subspecies was found, we used a guide to United States amphibians (not Florida-specific) to obtain the value for the species (Lannoo et al., 2005). See Supplementary Table 1 for full details on how each species' value for averages eggs/year was determined.

GLM Analysis

We analyzed the relationship between reproductive investment and species presence near roads using a generalized linear model in the program R v3.0.1 (R Development Core Team, 2013). The spatial unit of interest was section (east vs. west) and data from roadside and control trap subsamples were pooled for each section. In order to compare species with different numbers of individuals found, we converted data to proportions. Within each area, we summed the total number of individuals of a species

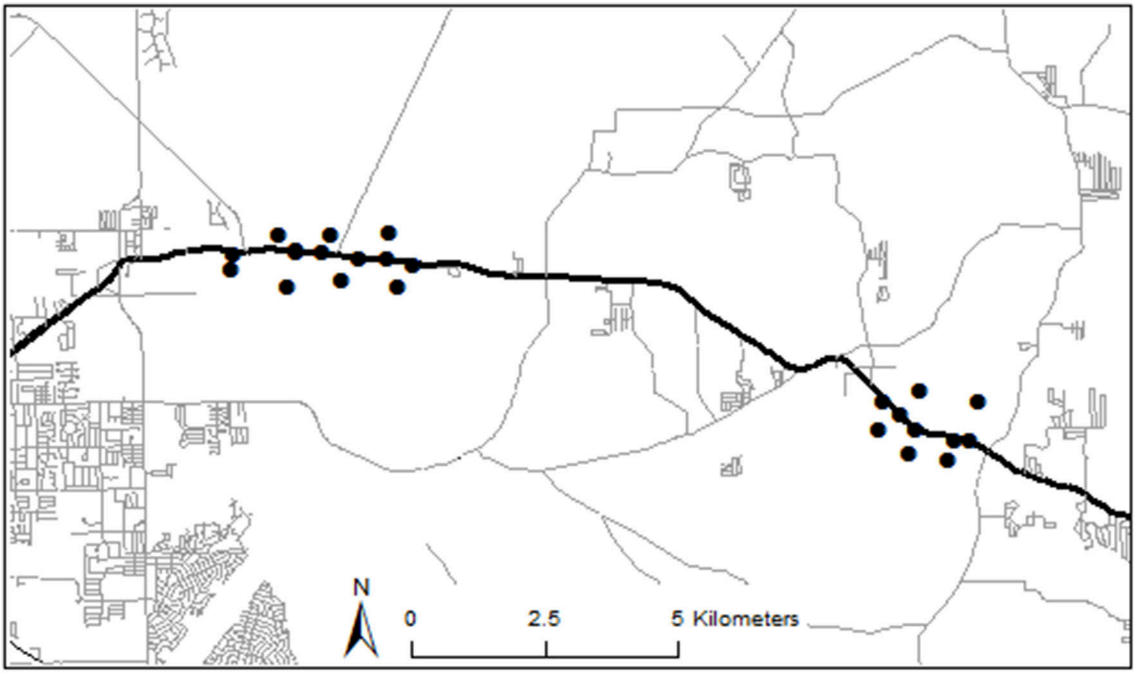

FIGURE 1 | Map of trap locations and nearby roads. State Road 40 is denoted by the thick black east-west line. Trap locations are denoted by black dots. Two dots indicating roadside traps in the western section appear to overlap on map, but they were separated by ≈100 m and were on different sides of the road. All paved roads within 5 km of study locations have annual average daily traffic volume (AADT) <2,000 cars/day. Unpaved roads and trails were omitted from this map for clarity.

found in roadside traps and control traps and calculated the proportion of individuals of that species found at roadside traps. Because our dependent variable was proportional, we tested for the effects of eggs/female/year (ln-transformed due to the wide range of values) and section using a binomial generalized linear model [glm(roadside proportion∼ ln(eggs/yr) + section, family = binomial)].

Model Weighting

We observed low numbers of certain species in our traps (**Table 1**), particularly treefrogs (*Hyla* spp.). Drift fences and bucket traps are not efficient methods for trapping treefrogs, since they can easily escape them (Dodd, 1991). Although this caveat applies to both roadside and control traps equally, low sample size could result in a calculated roadside proportion that does not reflect the true pattern of roadside vs. control abundance, simply by chance. However, the larger the total number of individuals observed, the more confident we can be that the roadside proportion of individuals is biologically significant. Therefore, in our model we weighted the data point for each species' calculated roadside proportion using the "weights" argument in the R function used to fit the generalized linear model, glm(). Including "weights" in the model ensures that data points with higher weights contribute more to final parameter estimates. We established a cutoff number of total observations below which the calculated proportion for a species became unreliable, and weighted those data points to have less influence than the rest of the data points.

We determined the cutoff using the following equation modified from Krebs (1999):

$$n = \frac{4\hat{p}(1 - \widehat{p})}{d^2}$$

where $n =$ the required sample size, $\hat{p} =$ the observed proportion, and $d =$ the desired margin of error. The most conservative sample size estimate is given when assuming that $\hat{p} = 0.5$ (Krebs, 1999), which is what we used. Using this formula, in order to achieve a margin of error no greater than ±10% ($d = 0.1$), the minimum sample size is 100 individuals. If a species total fell below the minimum sample size, it was weighted by dividing the number of individuals observed by 100. All species with 100 or more observed individuals received a weight of 1.

RESULTS

We observed 17 anuran species, with greatly varying reproductive rates (20–9,000 eggs/yr), in both roadside and control trap arrays (**Table 1**). Fourteen species were observed in both the west and east sections, though two species were observed only in the west section (Florida gopher frog, *Rana capito aesopus* and Florida chorus frog, *Pseudacris nigrita verrucosa*) and one species was only observed in the east section (little grass frog, *Pseudacris ocularis*; **Table 1**). Though the species we observed represent multiple families and different life histories (e.g., classic egg deposition in water vs. deposition on land, as in *Eleutherodactylus planirostris*), we observed a positive

TABLE 1 | List of anuran species encountered in trap surveys.

Species binomial	Common name	Family*	Eggs/yr	Sec.	Road prop.	Obs.	Weight
Acris gryllus dorsalis	Southern cricket frog	H	125	W	0.60	10	0.10
				E	0.23	197	1.00
Anaxyrus quercicus	Oak toad	B	210	W	0.19	16	0.16
				E	0.11	136	1.00
Anaxyrus terrestris	Southern toad	B	3,500	W	0.32	332	1.00
				E	0.18	56	0.56
Eleutherodactylus p. planirostris	Greenhouse frog	E	20	W	0.12	370	1.00
				E	0.18	40	0.40
Gastrophryne carolinensis	Eastern narrow-mouthed toad	M	800	W	0.33	650	1.00
				E	0.46	214	1.00
Hyla cinerea	Green treefrog	H	645	W	0.38	8	0.08
				E	0.00	2	0.02
Hyla femoralis	Pinewoods treefrog	H	275	W	0.25	12	0.12
				E	0.035	57	0.57
Hyla gratiosa	Barking treefrog	H	1,125	W	0.67	6	0.06
				E	0.20	5	0.05
Hyla squirella	Squirrel treefrog	H	650	W	0.14	28	0.28
				E	0.50	4	0.04
Pseudacris crucifer bartramiana	Southern spring peeper	H	100	W	0.25	8	0.08
				E	0.33	3	0.03
Pseudacris nigrita verrucosa	Florida chorus frog	H	117.5	W	0.71	7	0.07
Pseudacris ocularis	Little grass frog	H	100	E	0.00	10	0.10
Rana c. clamitans	Bronze frog	R	2,000	W	0.00	1	0.01
				E	0.00	1	0.01
Rana capito aesopus	Florida gopher frog	R	1,750	W	0.00	1	0.01
Rana grylio	Pig frog	R	9,000	W	1.000	1	0.010
				E	1.000	4	0.040
Rana sphenocephala	Southern leopard frog	R	1,000	W	0.694	111	1.000
				E	0.583	24	0.240
Scaphiopus holbrookii	Eastern spadefoot toad	S	150	W	0.081	604	1.000
				E	0.154	52	0.520

Eggs/ yr, The the average of the mean numbers of eggs per year from all sources listed; Road prop., the proportion of individuals found at roadside trap arrays; Obs., the total number of individuals (adults and sub-adults) found in trap arrays in 2012; Weight, the number of observations/100, used to weight the model. See Supplementary Material for source materials used to determine reproductive rate for each species.

**Family names: B, Bufonidae; E, Eleutherodactylidae; H, Hylidae; M, Microhylidae; R, Ranidae; S, Scaphiopodidae.*

correlation between number of eggs produced and roadside abundance (**Figure 2**, **Table 2**). In some cases, species with high reproductive rates had low abundance near roads and vice versa, but it appears that this may be a stochastic effect of low sample size and may not truly reflect the pattern within the species (this is reflected in low weighting of points with low sample size; **Figure 2**). There was a statistically significant effect of section (**Table 2**), however, the direction of the trend remains consistent between the two section (**Figure 2**).

DISCUSSION

Our results support the hypothesis that certain life history traits make species more vulnerable to the negative effects of roads (Rytwinski and Fahrig, 2012). In particular, we found that as anuran reproductive rate increases, anuran roadside abundance also increases, a pattern that has previously been noted in mammals (Rytwinski and Fahrig, 2011). Although our measure of the effects of roads on anuran demography is indirect, it provides a basis for predictions about the vulnerability of particular species to road mortality or population fragmentation. Given that our model did not explain all of the variance, there are undoubtedly factors at play that our study could not account for, including preferred habitat and behavioral patterns. For example, in our study Southern Leopard Frogs *Lithobates spenocephalus* (=*Rana spenocephala*) displayed a roadside proportion of 0.674, meaning that over half of the individuals caught were caught in roadside traps. This is probably explained by the fact that they often use roadside ditches to breed (Bridges and Semlitsch, 2001). However, knowing that some relationship between demography and roads exists is a critical piece of information for conservation biologists because it identifies certain species as particularly vulnerable to road effects, and therefore a higher priority for mitigation efforts.

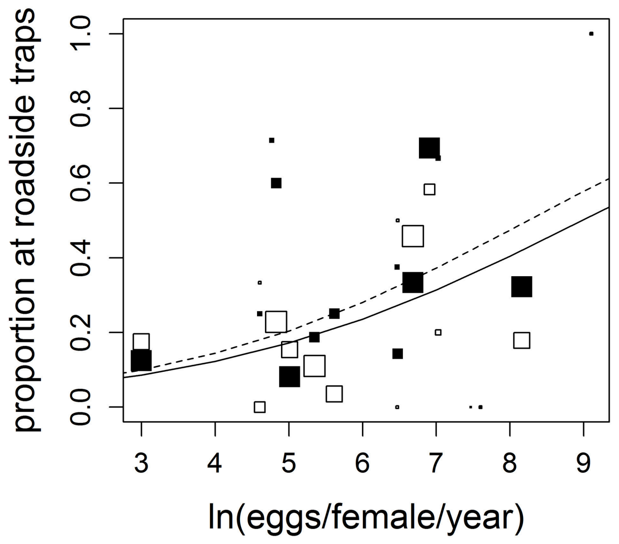

FIGURE 2 | Anuran species with higher reproductive rates were significantly more likely to be found in roadside traps. The black points represent species from the western section (solid line shows predicted model values) while the white points are from the eastern section (dashed line). Each point within each model represents one species. Species represented by smaller data points received lower weight in calculating model parameters because of lower sample size (see Section Materials and Methods). For full species names and sources for eggs/female/yr values, see Supplementary Material.

TABLE 2 | Anuran species that lay more eggs were found near SR 40 in higher proportions.

	Estimate	SE	*z*-value	Pr (>|z|)
Intercept	−3.57	0.218	−16.4	$<2e^{-16}$
ln (eggs/female/year)	0.399	0.0334	12.0	$<2e^{-16}$
Section (east)	0.236	0.105	2.24	0.0250

Summary table for the weighted glm (roadside proportion~ln(eggs/yr) + section, family = binomial) where "roadside proportion" is the proportion of adult frogs of a species found at roadside traps against the average number of eggs laid by a female of that species per year. Null deviance: 359.66 on 30 degrees of freedom. Residual deviance: 195.87 on 28 degrees of freedom.

However, a crucial piece of information is still needed to make the leap from identification of the correlation to effective mitigation. Though species with particular life history traits are known to show increased reduction of abundance near roads, the mechanism behind this reduced abundance remains unknown. There are two primary hypotheses that propose a mechanism: reduced abundance of species with low reproductive rates near roads might be caused either by (1) differential impacts of direct mortality (road-kill) or (2) selection for behavioral road avoidance (Fahrig et al., 1995; Eigenbrod et al., 2009; Rytwinski and Fahrig, 2012).

Populations of species with a low reproductive rate should experience a greater negative effect of road mortality than species with a high reproductive rate because the fewer offspring individuals have, the longer it will take for a population to recover from mortality events (Gibbs and Shriver, 2002; Rytwinski and Fahrig, 2011). It is also possible that species with a lower reproductive rate are inherently more "risk-averse." We might expect that such species would be more likely to behaviorally avoid roads than species that experience less selective pressure for road avoidance, explaining their reduced abundance near roads. Knowing whether animal populations are actively avoiding roads or are simply dying on them is critical to implementing an effective mitigation strategy. If a species avoids roads, then making a road more permeable with wildlife overpasses or underpasses will not necessarily eliminate the barrier effect of that road, because traffic noise or other cues may trigger the avoidance behavior.

Unfortunately, determining whether a population is succumbing to mortality or behaviorally avoiding roads is not easy. Previous studies of road avoidance behavior have been logistically difficult, requiring extensive field observation or satellite/radio-tracking. Therefore, much data on the distribution of animals relative to roads has been collected, but data on behavioral responses to roads is generally lacking (Rytwinski and Fahrig, 2012). While it is easy to collect roadkill data, this cannot always be extrapolated to make inferences about avoidance behavior unless more information about the size and distribution about the population overall is known.

The available literature provides some reason to expect that anurans could display road avoidance behavior. Amphibians in general appear to be highly susceptible to traffic mortality (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2012; Beebee, 2013), and anurans have been identified as highly vulnerable to road effects (Fahrig and Rytwinski, 2009; Rytwinski and Fahrig, 2012; Smith, 2012; Beebee, 2013), which could select for road avoidance behavior. There is growing consensus that risk-taking behavior and other aspects of animal "personality" have a genetic component (Stamps and Groothuis, 2010), which could allow selection for road avoidance. There may also be selective pressures for road avoidance besides road-kill: road noise has been shown to increase corticosterone levels in female Wood Frogs (*Lithobates sylvaticus*) and impair their ability to navigate to male breeding choruses (Tennessen et al., 2014). In addition, clear zones adjacent to roads are sometimes used by predators as efficient movement corridors or hunting areas (James and Stuart Smith, 2000; Colón, 2002; Laurance et al., 2004; Latham et al., 2011).

The available literature does not reject the possibility of road avoidance. Eigenbrod et al. (2008b) found that amphibian species richness was predicted better by the amount of habitat available without crossing a road (i.e., accessible habitat) than by total habitat. This indicates that some amphibian species may behaviorally avoid roads (Bouchard et al., 2009). Road mortality patterns also hint at species-specific, life-history-associated road avoidance: one study found that anuran roadkill was higher for a prolific breeder (Common Toads, *Bufo bufo*; ~1,500 eggs/yr, Beebee and Griffiths, 2000) than for a species with smaller clutch size (Fire-Bellied Toads, *Bombina bombina*; 80–300 eggs/yr, Lannoo et al., 2005) after controlling for how many animals attempted to cross the road using pitfall traps (Brzeziński et al., 2012). Fire-bellied toads approached the road less often than common toads and were hit less often when they did cross, indicating potential selection for avoidance of roads and also potentially vehicles. The one empirical study of anuran behavior near roads (Bouchard et al., 2009) observed no avoidance—all Northern Leopard Frogs (*Rana pipiens*), released near a road attempted to cross it—but this may not be a universal response across anuran species. Since, Northern Leopard Frogs have a high reproductive rate (645–7,648 eggs/clutch; Lannoo et al., 2005), the population may be less susceptible to road effects, making individuals less likely to avoid roads.

Amphibian-specific road crossings (ecopassages) are becoming more commonplace and may help to mitigate the negative effects of roads on anurans (Dodd et al., 2004). However, if roads elicit behavioral avoidance in some species, ecopassages will not be enough to mitigate their impact. In such cases it may be necessary to create new breeding ponds that can be reached without crossing roads (Beebee, 2013), or even to seasonally close roads to traffic (Jackson et al., 2015). Considering the rapid extinction of many amphibian species worldwide (e.g., Houlahan et al., 2000; Stuart et al., 2004), identifying threats to vulnerable species and the best way to mitigate those threats will be critical for maintaining amphibian biodiversity into the future.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the University of Central Florida Institutional Animal Care and Use Committee under protocol #11-37W and conducted under Scientific Collecting Permit #LSSC-11-00092.

AUTHOR CONTRIBUTIONS

MG analyzed the data and wrote the bulk of the manuscript. DS and RN were principal investigators on the project that generated the data and co-wrote and edited the manuscript. All authors have given approval of the final version of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fevo.2017.00065/full#supplementary-material

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Effectiveness of Road Mitigation for Common Toads (*Bufo bufo*) in the Netherlands

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Roads and traffic may have major impacts on amphibian populations, primarily as a result of amphibian road mortality. A variety of measures have been developed to prevent road mortality of amphibians, such as the construction of fences to keep the animals off the road and amphibian tunnels to provide them a safe passage. We carried out a capture-mark-recapture study to evaluate the performance of two tunnels and permanent drift fences for common toads at a local road in the Netherlands. We found that of the marked toads only 31% used the tunnels to cross the road. We assessed four possible explanations for the fact that a proportion of the toads did not use the tunnels: for toad groups that used the tunnels, as compared to toad groups that did not use the tunnels, (1) the mean distance between the location of first capture and the nearest tunnel was significantly smaller; (2) the mean movement distance along the fence was significantly larger; (3) the number of toad groups that walked in the wrong direction after encountering the drift fence was lower; (4) the mean number of nights between first and last capture of the toad group was significantly higher. Over all study years 28% of the migrating toads—marked and unmarked—that attempted to cross the road ended up on the road pavement, despite the mitigation. Migrating population numbers decreased with about 75% after the mitigation measures were installed. We emphasize that better baseline studies on where toads cross before mitigation and improved knowledge on effects of tunnel design and the distances the animals move along a drift fence are vital to mitigate road impacts properly and maintain viable toad populations. We recommend to base tunnel densities on the mean movement distance of the toads that move only small distances and spent relatively little time along the drift fence, install drift fences that go well beyond the location where toads cross the road, take appropriate measures at entrance roads and at fence ends and consider alternatives to tunnels and fences, such as the creation of breeding waters on both sides of the road.

Keywords: habitat fragmentation, road mortality, road mitigation, amphibian tunnel, amphibian fence, toad, population effect

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INTRODUCTION

Roads and traffic have major impacts on animal populations (Forman et al., 2003; van der Ree et al., 2015). The impacts of roads and traffic on amphibian populations is primarily the result of amphibian road mortality (Fahrig et al., 1995; Carr and Fahrig, 2001; Hels and Buchwald, 2001; Gibbs and Shriver, 2005; Andrews et al., 2008; Glista et al., 2008; Beebee, 2013). This is particularly obvious in species such as the common toad (*Bufo bufo*), that usually migrate in large numbers between their wintering and breeding habitat and need to cross roads during these movements (see van Gelder, 1973; Santos et al., 2007; Cooke, 2011). A variety of measures have been developed to prevent road mortality of amphibians, of which the construction of fences to keep the animals off the road and amphibian tunnels to provide them a safe passage are the most common ones (Iuell et al., 2003; Schmidt and Zumbach, 2008; Jackson et al., 2015). Although such measures are frequently applied across the world, only a few studies have evaluated their effectiveness in reducing road-kill and facilitating safe movements across roads (Woltz et al., 2008; see overview in Glista et al., 2009; Niemi et al., 2014).

High road-kill numbers among common toads have been observed on a local road near Ede city in the central parts of Netherlands. Road-kill occurred particularly during spring migration, as the animals had to cross the road to migrate from their wintering habitat (south of the road) to their breeding ponds (north of the road). Until 2010 volunteers put up temporary drift fences and pitfall traps to capture the migrating animals and transport them manually across the road. In 2010 the temporary measures were replaced by two tunnels and permanent drift fences. During the spring migrations of 2011 and 2012 use of the tunnels by toads was frequently observed. However, it was also noted that not all toads were successful in finding the tunnels. In addition, a proportion of the migrating toads were still killed by traffic despite the mitigation. Further, no inferences could be made on the effectiveness of the mitigation on preserving the toad population as population estimates after mitigation were lacking. Our objective is to evaluate the performance of these mitigation measures for common toads and assess (1) what proportion of the toads that approach the road make use of the tunnels, (2) the possible reasons that some of the toads do not use the tunnels, (3) what proportion of the toads that approach the road end up on the tarmac, despite the mitigation, and (4) whether size of the migrating toad population differs before and after the installation of the mitigation measures.

METHODS

Study Site

Our study site is a 1-km road stretch of a local road—the "Horalaan"—on the outskirts of the city of Ede, The Netherlands. The road provides access to a business site and private properties situated in a forested area. The road is paved, five meters wide and traffic volume is relatively low (<5,000 vehicles/day). The road crosses mixed-forests on sandy soils, which are poor in nutrients and deposited during glacial periods. The area is about 17.5–50 m above sea level. These forests host one of the four largest known common toad populations in the country (Ottburg and van Blitterswijk, 2009). Land habitat of the toads is mainly found south of the road. The vegetation exists here of Oak-Beech and Oak-Birch forests and plantations of Pine (e.g., *Pinus sylvestris*) and Spruce (e.g., *Picea abies*). Dominant tree species are Common oak (*Quercus robur*), Sessile oak (*Quercus petraea*), Beech (*Fagus sylvatica*), Downy birch (*Betula pubescens*), and Silver birch (*Betula pendula*). The undergrowth mainly consists of Alder buckthorn (*Rhamnus frangula*), Elder (*Sambucus nigra*), Honeysuckle (*Lonicera periclymenum*), Calluna (*Calluna vulgaris*), Bracken (*Pteridium aquilinum*), Blueberry (*Vaccinium myrtillus*), and Blackberry (*Rubus fruticosus*). Breeding habitat is situated north of the road and consists of three ponds. Distance between road and ponds varies between 140 and 200 m. The ponds are up to 1.5 m deep and are, respectively, about 220, 700, and 720 square meters in size. The aquatic vegetation consists mainly of Western waterweed (*Elodea nuttallii*), Mare's tail (*Hippurus vulgaris*), Fringed waterlily (*Nymphoides peltata*) and the invasive New Zealand pigmyweed (*Crassula helmsii*). The pond banks are about 3–5 m wide. The vegetation on the banks consists mainly of Willow (*Salix* spec.), Blackberry (*Rubus fruticosus*), Common rush (*Juncus effusus*), Common cattail (*Typha latifolia*), Slender tufted-sedge (*Carex acuta*), Purple loosestrife (*Lythrum salicaria*), and Reed (*Phragmites australis*). The ponds were excavated in 2010 because, at that time, four existing breeding waters were lost due to urban development. The new ponds were situated about 100 meters south of the existing breeding waters.

Road Mitigation Measures

In 2010 two amphibian tunnels and permanent drift fences were installed (**Figure 1**). The tunnels are two-way concrete tunnels (type ACO), 8.6 m long, 0.50 m wide, 0.30 m high, with an open roof (grid). The tunnels are situated about 100 m apart in the central part of the mitigated road stretch. The drift fences are smooth black barriers, 0.40 m high and made of high density poly-ethylene. The fence on the south side of the road is 900 m long. The fence on the north side of the road is 1,000 m long. The fence ends include a 25 m-long drift fence perpendicular to the road. Where access roads cross the fences, small cattle guards have been installed to prevent the toads from accessing the road corridor.

Data Collection

In 2013, 2014, and 2015 we carried out a capture-mark-recapture study. We installed 36 pitfall traps, 25 m apart,

FIGURE 1 | Amphibian tunnel and drift fence at the study site. Photo: F. Ottburg.

along the drift fence south of the road. Pitfall traps were also installed at both tunnel exits, north of the road (**Figure 2**). Immediately after the start of spring migration toad surveys were conducted (1) along the drift fence on the south side of the road, (2) on the road and (3) at the northern tunnel exits.

During the surveys along the drift fence all toads that were captured in the pitfall traps or that were found moving along the fence were individually marked. For marking we used a small sticker with a unique number (hereafter referred to as "ID number") that was attached to the back of the toad with the help of a biodegradable glue (**Figure 3**). If toads were in amplexus, i.e., the mating embrace during which eggs will be shed into the water by the female and there fertilized by the male, we only attached a sticker to the back of the male toad. Based on re-sightings of marked toads in the breeding ponds, we estimate that the markings stayed on for at least 1 week. After marking we released the toads on the same spot where they had been found along the fence or at a distance of 1–2 m from the pitfall trap where they had been captured. We recorded for each captured toad, date, time of capture, sex, life stage (sub-adult, adult), and capture location. Capture location was registered as either pitfall trap number (1–36) or fence-stretch indication (1–2, 2–3, 3–4, etc.). During data entry these location descriptions were transformed into distances (in m), measured from the most western pitfall trap (number 1). If the capture location was in between two pitfall traps, the central spot of that particular fence stretch was used. This approach results in a spatial resolution of 1 m for the pitfall traps (exact locations) and 25 m for toads that were captured in between the pitfall traps. In 2013 we used all 36 pitfall traps. In 2014 and 2015 we used trap 1, 5, 9, 13, 17, 21, 25, 29, 33, and 36. The others were closed, aiming to reduce the number of trap encounters for a migrating toad during one night.

During the road surveys all toads found on the road, dead or alive, were counted. We recorded date, time of discovery, sex and life stage (if recognizable), capture location and, if present, their ID number. For capture location we distinguished four 250 m-road sections (A–D; see **Figure 2**). Dead toads were removed from the pavement to avoid double counts. Living toads were collected and released in one of the breeding ponds.

During the surveys of the pitfall traps at the tunnel exits, all toads were counted. We recorded date, time of capture, sex, life stage, capture location (tunnel 1 or 2) and, if present, their ID number. All toads were collected and released in one of the breeding ponds.

The surveys took place in the morning (6:00–11:00), incidentally in the afternoon (14:00–18:00), and in the evening (19:00–24:00), on days that weather conditions were assumed favorable for toad activity (air temperature >8°C and preferably some rain). During one survey the pitfalls, drift fence, and road was checked two times. The second check ended more or less when the toads became inactive, i.e., were no longer found moving along the drift fence. That "inactivity" could be judged adequately, is supported by the observation that, over all study years, only about 5% of all toads captured along the drift fence, have been captured during morning surveys. The survey period ended when no more toads were found along the drift fence for at least five consecutive days. **Table 1** provides an overview of the survey characteristics per study year.

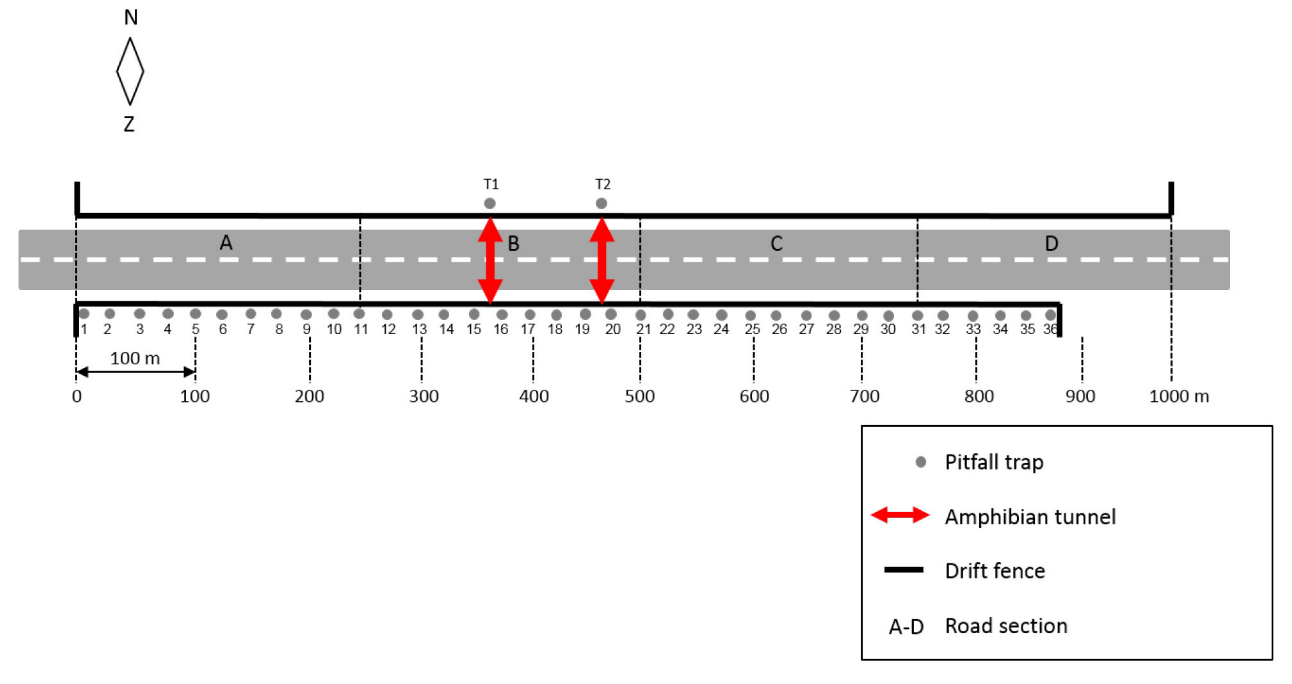

FIGURE 2 | Schematic overview of study site and experimental set-up.

FIGURE 3 | Toads with marking moving along the drift fence. Photo: F. Ottburg.

Data Analysis

The proportion of toads that approach the road and make use of the tunnels was estimated by dividing the number of marked toads that did use the tunnels by the total number of marked toads. Herein "marked toads" is defined as the sum of (1) all individually marked toads (male or female), (2) all males that are part of a marked amplexus and (3) all females that are part of a marked amplexus. A crossing index (CI) was calculated through dividing the number of marked toads that did use the tunnels by the number of marked toads that did not use the tunnels. This implies that if $CI = 1$, the number of toads that did or did not use the tunnels is equal; if $CI < 1$, the number of toads that used the tunnels is lower than the number of toads that did not; if $CI > 1$, the number of toads that used the tunnels is higher than the number of toads that did not. A road permeability index (RPI) was calculated through dividing the number of marked and unmarked toads that did use the tunnels by the total number of toads that attempted to cross the road to reach the breeding ponds. This total number was estimated by adding up (1) the number of marked toads, (2) the number of unmarked toads on the road, dead or alive, and (3) the estimated number of missed toads along the drift fence, calculated through multiplying the number of unmarked toads in the pitfalls at the tunnel exits with $1/CI$.

To explore the reasons why some of the toads do not use the tunnels we tested five hypotheses. If toads that do not use the tunnels are compared with toads that do use the tunnels, we expected the first to (1) arrive at the drift fence further away from a tunnel and, hence, these toads will have to cover more distance; (2) move less distance along the fence; (3) pass tunnels more often without using them; (4) walk more often in the wrong direction, i.e., away from the tunnels, after encountering the drift fence; (5) spend less time along the drift fence. Our null hypothesis was that there is no difference in these variables for toads that do and do not use the tunnels. In testing these hypotheses we used "toad group" as unit. A toad group is either an individual toad or two (or three) toads in amplexus. We chose this approach—instead of using "toad" as unit—because the movements of the toads that are part of an amplexus are not independent.

We compared the mean distance between the location of first capture and the nearest tunnel (test 1), the mean movement distance along the fence, derived from the location of first capture and the location of last re-capture (test 2), the number of toad groups that walked past a tunnel (test 3), the number of toad groups that walked in the wrong direction after encountering the drift fence (test 4), and the mean number of nights between first and last moment the toad group was captured (test 5) for toad groups that did and did not use the tunnels over all study years. Marked toad groups found on the road ($n = 2$) were excluded from the analysis as the spatial resolution of the road survey data was insufficient. Marked toad groups for which the location and time of first capture was not known ($n = 10$), were excluded from the analyses as for these animals no inferences on movement distances or time spent along the drift fence could

TABLE 1 | Survey characteristics for each study year.

Survey characteristics	2013	2014	2015
Start date	March 5	February 20	February 21
End date	April 18	April 8	April 13
Number of survey days	18	34	52

be made. Marked toad groups that were not recaptured ($n = 243$) or did not move between first capture and consecutive recaptures ($n = 14$) were excluded from the analysis in test 4 as no inferences could be made on their direction of movement. In test 2, the calculated mean for toad groups that did not use the tunnels can be seen as a minimum value as toad groups that are captured only once result in a movement distance of 0 m, while it is likely that the animals did move along the fence for a while but left it before the next survey was carried out. In test 1, 2, and 5 we tested for significance between the means with the use of the non-parametric Mann-Whitney *U*-Test ($p < 0.05$ significance level) as normal distribution of the data could not be assumed and the number of possible values was limited. We used the Chi-square test of association ($p < 0.05$ significance level) to test for independence between the categorical variables "walking past one or more tunnels" and "eventual use of the tunnels" as well as "toad group movement direction after encountering the drift fence" and "eventual use of the tunnels." We explored whether sex affected tunnel use probability for toads that moved along the drift fence individually by logistic regression. The same analysis was performed to explore whether toads in amplexus were more likely to use the tunnels than individual toads.

The proportion of the migrating toads that ended up on the road was estimated by dividing the number of toads that were found on the road—dead or alive—by the estimated population size (see below) per study year. These proportions were averaged to calculate an estimate over all study years.

For the years 2013–2015, the size of the toad population that had to cross the road to reach the breeding ponds, was estimated by adding up (1) the number of toads found along the drift fence that were not marked but immediately transported to the breeding ponds, (2) the number of marked toads, (3) the number of unmarked toads on the road, dead or alive, and (4) the estimated number of missed toads along the drift fence. The first group of toads, which were immediately transported to the breeding ponds, concerned toads that were discovered by volunteers prior to the start date of the surveys. The estimated number of missed toads along the drift fence was calculated through multiplying the number of unmarked toads in the pitfalls at the tunnel exits with 1/CI. Estimates of the size of the toad population that had to cross the road to reach the breeding ponds in the pre-mitigation situation were derived from a previous survey, carried out in 2007 and 2008 (Ottburg and van Blitterswijk, 2009). In this earlier survey all toads that were found along temporary drift fences—which were of the same length as the later as part of the mitigation installed permanent drift fences—and on the road were counted by volunteers after which the animals were captured and released in the breeding ponds. Similar to our study, these surveys lasted for the entire period of spring migration, which was, respectively 28 days (2007) and 59 days (2008).

RESULTS

Over all study years a total of 722 toads were marked (**Table 2**), either individually (43%) or as part of an amplexus (57%). 61% of the marked toads were males, 38% were females, and for 1% sex was not identified. All marked toads were adults. Of the marked toads 31% used the tunnels to cross the road, 68% were not detected using the tunnels to get across, and 1% ended up on the road. For all years together the CI is 0.46, indicating that the number of toads that did use the tunnels is less than half the number of toads that did not. The RPI is 0.26, indicating that only about one quarter of all toads that attempted to cross the road, managed to find and use the tunnels. Logistic regression showed that male toads were more likely to use the tunnels than female toads ($B = -1.13$, s.e. $= 0.155$, $p < 0.001$). Twenty four percent of all marked males, excluding the ones that were part of an amplexus, were detected to use the tunnels vs. 8% of all marked females. Amplexuses were more likely to use the tunnels than individually moving toads ($B = -0.95$, s.e. $= 0.156$, $p < 0.001$). Twenty eight percent of all marked amplexuses were detected to use the tunnels vs. 20% of all marked individual toads (male or female).

Over all study years a total of 504 toad groups were marked. Of these, 118 used the tunnels and 386 did not. The toad groups were not evenly distributed over the drift fence; about 90% of all toad groups were first captured along the 400-m road stretch between kilometer-post 0.4 and 0.8 (**Figure 4**).

The mean distance between the location of first capture and the nearest tunnel was significantly smaller ($U = 16608$, $z = 4.45$, $P < 0.001$) for toad groups that did use the tunnels as compared to toad groups that did not use the tunnels (**Table 3**). On average toad groups that used the tunnels encountered the drift fence almost 50 m closer to the nearest tunnel than toad groups that did not use the tunnels.

The mean movement distance along the fence was significantly larger ($U = 5993$, $z = 12.12$, $P < 0.001$) for toad groups that did use the tunnels as compared to toad groups that did not use the tunnels (**Table 4**). On average toad groups that used the tunnels moved 120 m further along the drift fence than toad groups that did not use the tunnels.

The number of toad groups that walked past a tunnel was higher for toad groups that did use the tunnels as compared to toad groups that did not use the tunnels (**Table 5**). On average 12% of the toad groups that eventually used the tunnels first walked past a tunnel vs. 2% of toad groups that did not use the tunnels. A chi-square test showed there was an association between tunnel use and walking past one or more tunnels ($\chi^2 = 18.86$, d.f. $= 1$, $P < 0.001$).

For 247 toad groups the movement direction along the drift fence is known after first capture, of which, respectively 129 and 118 toad groups did and did not use the tunnels eventually. The number of toad groups that walked in the wrong direction after encountering the drift fence was lower for toad groups that did use the tunnels as compared to toad groups that did not use the tunnels (**Table 6**). On average 13% of the toad groups that eventually used the tunnels first walked in the wrong direction vs. 37% of toad groups that did not use the tunnels. A chi-square test showed there was an association between tunnel use and the direction of movement after encountering the drift fence ($\chi^2 = 19.46$, d.f. $= 1$, $P < 0.001$).

TABLE 2 | Number and proportion of marked toads that did and did not use the amphibian tunnels per study year and over all study years.

	2013		2014		2015		2013–2015	
	N	%	N	%	N	%	N	%
Marked toads	481	–	118	–	123	–	722	–
Adult males	285	59	70	59	86	70	441	61
Adult females	192	40	48	41	37	30	277	38
Adults, sex unknown	4	1	0	0	0	0	4	1
Number of amplexuses	139	–	36	–	29	–	204	–
Marked toads that used the tunnels	162	34	37	31	28	23	227	31
Marked toads that did not use the tunnels	319	66	80	68	94	76	493	68
Marked toads that ended up on the road	0	0	1	1	1	1	2	<1

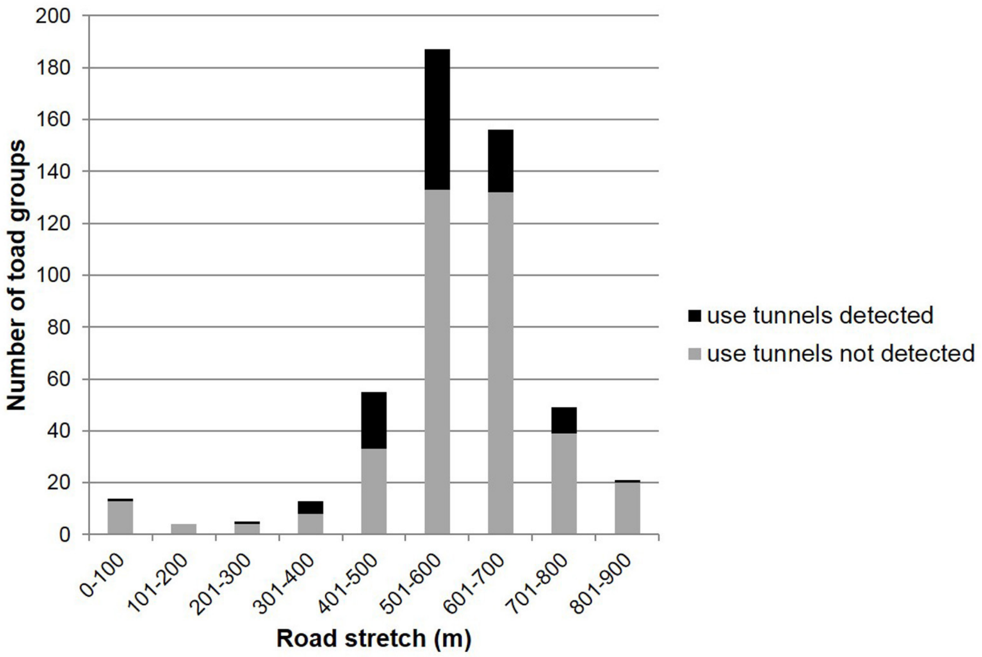

FIGURE 4 | Distribution of toad groups that eventually did and did not use the tunnels along the drift fence during first capture over all study years.

The mean number of nights between first and last capture of the toad group was significantly higher ($U = 10433$, $z = 8.91$, $P < 0.001$) for toad groups that did use the tunnels as compared to toad groups that did not use the tunnels (**Table 7**). On average, toad groups that used the tunnels spent two days more along the drift fence than toad groups that did not use the tunnels.

Over all study years 28% of the migrating toads—marked and unmarked—that attempted to cross the road ended up on the road pavement, despite the mitigation (**Figure 5**). Of these 20% were found dead and 80% were found alive. The number of toads per road section steeply increases from west to east with <50 toads in section A to over 250 toads in section D (**Figure 5**).

Migrating population numbers decreased with about 75% after the mitigation measures were installed (**Figure 6**). Before mitigation the toad population that had to cross the road to reach the breeding ponds, was estimated at about 3,000 individuals. After mitigation, this population size was estimated at about 800 individuals.

DISCUSSION

We found that a relatively small proportion of the toads that approached the road managed to get across via the two amphibian tunnels. The main reason behind this seems to be the low tunnel density (2.2/km) which may present too large a distance for most toads to cover. Mean movement distance along the fence for all toad groups is about 60 meters, while the mean distance to the nearest tunnel after encountering the drift fence is about 160 meters. Installing more tunnels will likely allow more toads to reach and use them to get across.

We found a relatively low CI and RPI. Both indices will be underestimated if toads that used the tunnels successfully were

TABLE 3A | Mean distance (D_{mn}) and standard deviation of the mean (sd) between location of first capture and nearest tunnel per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	D_{mn}	sd	D_{mn}	sd	D_{mn}	sd	D_{mn}	sd
All marked toad groups	157	92	160	108	155	108	157	98
Marked toad groups that used the tunnels	119	81	111	86	143	123	121	88
Marked toad groups that did not use the tunnels	169	92	175	111	157	106	168	98

TABLE 3B | Median distance (D_{md}) and interquartile range (IQR) between location of first capture and nearest tunnel per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	D_{md}	IQR	D_{md}	IQR	D_{md}	IQR	D_{md}	IQR
All marked toad groups	140	118	190	150	90	150	140	138
Marked toad groups that used the tunnels	153	125	190	150	90	150	160	150
Marked toad groups that did not use the tunnels	103	113	90	165	90	133	103	125

not captured in the pitfall traps at the tunnel exits or when marked toads lost their markings along the way. As the traps at the tunnel exits covered the full width of the tunnels, it was impossible for the toads to exit the tunnels on the north side without being captured. Some toads may have turned around after encountering the trap, however, we expect these numbers to be low, as a sudden drop into the traps was created at the tunnel exits. Although the loss of markings cannot be excluded, we expect this to have happened only occasionally. No lost markings were found during the surveys along the drift fence, in the tunnels or in the pitfall traps. Moreover, many marked toads were observed in the breeding ponds, more than a week after they had been marked along the drift fence.

Besides the distance between tunnels, design features of the tunnels may play a role in tunnel acceptance and use. If, for example, the tunnels are perceived by the toads as too small, too long or with an inhospitable micro-climate, they may avoid them. The fact that a proportion of the marked toad groups walked past a tunnel may indicate the existence of such an effect. However, as only 14% of all marked toad groups have been found to walk past a tunnel, we conclude that design features can only partially explain the low crossing rates. To gain more insight in the effect of tunnel design we recommend to carry out a study with the use of motion-triggered camera traps. This will allow to study the behavior of individual toads that arrive at the tunnel entrances and will help to assess what proportion of the toads that enter a tunnel pass it fully and what proportion turns around somewhere halfway.

The data show that the behavior of individual toads differs considerably. At one side of the spectrum there are toads that move large distances and spent considerable time along the drift fence in an attempt to get across the road ("runners"). On the other side there are toads that move only small distances and spent relatively little time along the drift fence ("strollers"). The runners are more successful in using the tunnels, even despite the fact that they walk past tunnels more often than the strollers. In decisions on the number of tunnels needed, the demands of the strollers should preferably be leading, as they have the greatest demands in respect to the maximum distance between tunnels.

If desired tunnel density is based on the mean movement distance (about 40 m; **Table 4**) of the toad groups that did not use the tunnels, 23 tunnels should be installed along the 0.9 kilometer-road stretch (tunnel density: 25/km). It should be noted, however, that the mean movement distance of the toad groups that did not use the tunnels is likely underestimated. Many non-crossing toad groups ($n = 202$; 52%) were not recaptured and hence their movement distance was set as 0 m. It is likely, however, that these toads did move along the fence after being released, but left the fence before the next survey was carried out. Hence, we recommend the use of the mean movement distance for all toads (about 60 m; **Table 4**) as rule-of-thumb for the maximum distance between tunnels. In that case 15 tunnels should be installed along the 0.9 kilometer-road stretch (tunnel density: 16.6/km). For future studies, we recommend to increase chances for recapturing toads, carrying out additional checks during both the morning and evening surveys, as this may reduce the number of "0 m movements." We also recommend increasing the spatial resolution of the data through applying 1 m-markings on the drift fence. This will allow for better inferences about both capture locations and distances moved.

Another issue in relation to the desired number of tunnels is that the toads did not arrive evenly distributed over the fence length. About 90% of all toads arrived within a stretch of 400 m (**Figure 4**). If desired tunnel density is based on the mean movement distance of all toad groups (60 m) and is limited to

TABLE 4A | Mean movement distance (M_{mn}) and standard deviation of the mean (sd) along the drift fence per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	M_{mn}	sd	M_{mn}	sd	M_{mn}	sd	M_{mn}	sd
All marked toad groups	73	110	51	82	43	92	64	103
Marked toad groups that used the tunnels	160	141	130	96	163	148	156	135
Marked toad groups that did not use the tunnels	44	78	27	61	18	48	36	71

TABLE 4B | Median movement distance (M_{md}) and interquartile range (IQR) along the drift fence per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	M_{md}	IQR	M_{md}	IQR	M_{md}	IQR	M_{md}	IQR
All marked toad groups	14	113	0	90	0	10	0	100
Marked toad groups that used the tunnels	0	63	0	0	0	0	0	50
Marked toad groups that did not use the tunnels	128	131	160	165	100	188	125	138

TABLE 5 | Number of toad groups that walked past a tunnel per toad group and per study year and over all study years.

	2013	2014	2015	2013–2015
All marked toad groups	21	1	1	23
Marked toad groups that used the tunnels	12	1	1	14
Marked toad groups that did not use the tunnels	9	0	0	9

TABLE 6 | Number of times that the wrong direction was chosen after encountering the drift fence per toad group and per study year and over all study years.

	2013	2014	2015	2013–2015
All marked toad groups	57	7	5	63
Marked toad groups that used the tunnels	12	2	1	15
Marked toad groups that did not use the tunnels	39	5	4	48

this 400 meter-road stretch where most toads arrive, in total 7 tunnels should be installed. Hence, for future mitigation projects we recommend to explore the spatial distribution of locations where toads cross the road in detail prior to the installation of the mitigation measures. This will allow for tailor-made solutions and decrease the cost-benefit ratio.

Over all study years about 28% of the migrating toads ended up on the road. Most of these toads were still alive when found, but it seems reasonable to assume that a large proportion of them would have been killed eventually if they had not been captured and moved to the ponds as part of our study. Three possible explanations for these relatively high number of toads in the road corridor are (1) toads walk around the fence ends, (2) toads are able to jump over the drift fence, (3) toads use breaches in the drift fence, and (4) toads are able to pass the cattle guards at side roads. The first explanation certainly applies as we frequently observed toads entering the road surface beyond the fence ends during our surveys. This particularly occurred beyond the eastern fence end, which seems reflected in the high numbers of toads found in road section D (**Figure 5**). The southern drift fence is here 100 m shorter than the northern one, which means that toads that surpass the southern barrier will encounter the fence on the north side of the road and likely end up wandering on the pavement between the two fences. Some toads also appeared to be able to pass the cattle guards at five side roads, which are all located in road sections B and C. During our surveys we repeatedly observed how toads were able to balance themselves on the grid and enter the road corridor. No evidence has been found for toads jumping over the fence or using breaches in the fence. Before the surveys took place the drift fence was carefully checked for breaches and, if needed, repaired. Both before and during the surveys conditions that could possibly help toads jumping the fence, e.g., fallen branches or accumulation of litter, were removed. **Table 2** shows that a relatively small proportion of marked toads ended up on the road. One possible explanation is that the markings can be easily missed after marked toads have been killed on the pavement, which may imply that the number of marked toads on the road is underestimated. Another explanation is that the number of toads that enter the road corridor at the fence ends is considerably higher than the number of toads that enter the road across the cattle guards at side roads. At the fence end the proportion of entering toads that is marked is likely lower, as these not only include toads that surpass the fence but also toads that approach the road beyond the fence end, where no capturing took place. The latter explanation is supported by the relatively high number of toads found in road section D (**Figure 5**). For future mitigation projects we recommend (1) apply drift fences over a length that goes well beyond the road stretch where the toads cross, as crossing locations may shift between years, (2) apply drift fences on both sides of the road that are equal in length, and (3) install toad-proof cattle guards in the road at both fence ends and at all side roads to prevent toads from entering the road corridor between the fences (**Figure 7**).

TABLE 7A | Mean number of nights (N_{mn}) and standard deviation of the mean (sd) between first and last capture per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	N_{mn}	sd	N_{mn}	sd	N_{mn}	sd	N_{mn}	sd
All marked toad groups	2.3	1.5	2.8	3.6	2.0	2.1	2.3	2.11
Marked toad groups that used the tunnels	3.3	1.6	6.4	5.1	3.3	3.4	3.8	2.90
Marked toad groups that did not use the tunnels	1.9	1.3	1.6	1.9	1.8	1.6	1.8	1.49

TABLE 7B | Median number of nights (N_{md}) and interquartile range (IQR) between first and last capture per toad group per study year and over all study years.

	2013		2014		2015		2013–2015	
	N_{md}	IQR	N_{md}	IQR	N_{md}	IQR	N_{md}	IQR
All marked toad groups	2.0	2.0	1.0	1.8	1.0	1.0	1.0	2.0
Marked toad groups that used the tunnels	1.0	1.0	1.0	0.8	1.0	0.0	1.0	1.0
Marked toad groups that did not use the tunnels	3.0	2.0	4.0	9.5	2.0	2.3	3.0	3.0

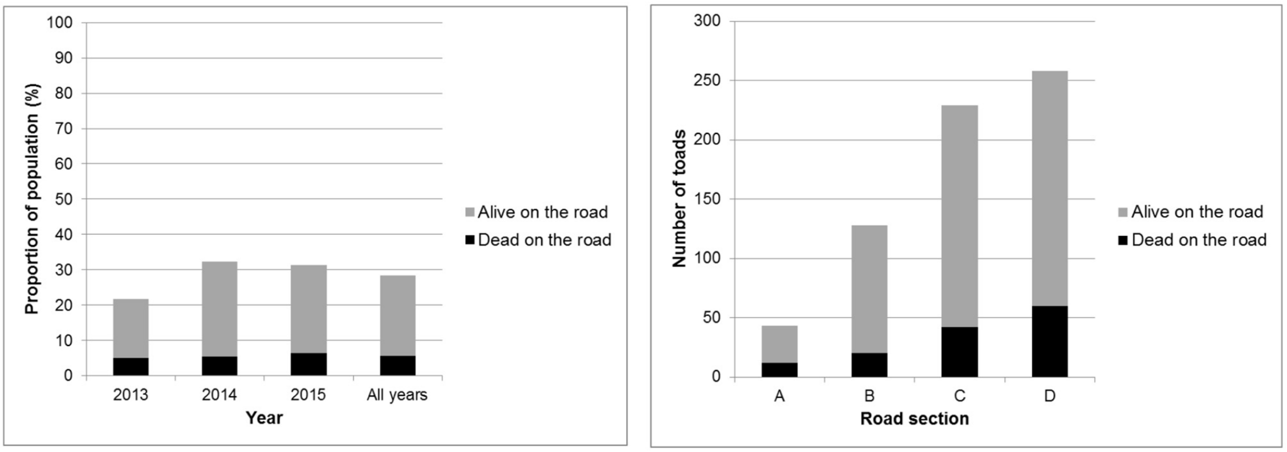

FIGURE 5 | Proportion of the migrating toads that were found—dead or alive—in the road corridor (left) and distribution of these toads over the road sections (right).

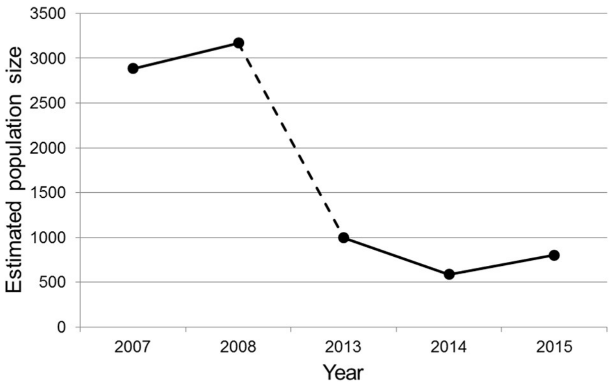

FIGURE 6 | Trend in population size before (2007–2008) and after (2013–2015) the road mitigation measures were installed.

The toad population that had to cross the road to reach the breeding ponds, decreased significantly after the mitigation measures were installed (**Figure 6**). One explanation may be the removal of the historic breeding waters, as the total size of all breeding waters was reduced from about 6,775 m^2 in 2007–2008 (pre-mitigation) to 1,640 m^2 in 2013–2015 (post-mitigation). Another plausible explanation for this steep decline is the relatively small number of toads that manages to get across the road and take part in breeding. Even more so if we assume that something similar applies to the migration of adults and juveniles back to the land habitat after breeding. Considering that—simultaneously with the installation of the mitigation—land habitat north of the road was lost or became inaccessible for toads due to city expansion, one could expect increasing numbers of toads that migrate to land habitat south of the road. However, the opposite has been observed. Instead of, or besides, building more tunnels to increase the proportion of toads that make it across the road and take part in breeding, the creation of breeding waters south of the road may be considered. Currently, toads that fail to use the tunnels will have no part in breeding as no ponds occur south of the road. Additional tunnels will increase the likelihood that toads will get across safely, but there may always be a proportion

FIGURE 7 | Example of a toad-proof cattle guard **(left)** and one that can still be bridged by toads **(right)**. The opening between the road and the first steel bar in the toad-proof cattle guard is 6 vs. 4 cm in the one that can be bridged by toads. Photos: F. Ottburg.

of the migrating toads that do not manage to reach or use the tunnels. With the creation of ponds south of the road these toads are offered an alternative breeding place. When such a measure is applied, we recommend the careful monitoring of the acceptance and use of the new ponds by toads and evaluate the effectiveness of the measure to increase participation in breeding and eventually population numbers.

CONCLUDING REMARKS

During spring migration the road mitigation measures at our study site provide a safe passage across the road for a relatively small proportion of the migrating toads. Most toads do not manage to get across through the tunnels and consequently take no part in breeding. In addition toads frequently end up on the road where they run the risk of being killed by passing cars. This all seems to have seriously affected population numbers and may even threaten the survival of the population. Our research emphasizes that better baseline studies on where toads cross before mitigation and improved knowledge on the distances the animals move along a drift fence are vital to mitigate road impacts properly and maintain viable toad populations. We recommend to base tunnel densities on the mean movement distance of the toads that move only small distances and spent relatively little time along the drift fence, and install drift fences that go well beyond the location where toads cross the road. At entrance roads and at fence ends tailor-made measures are needed to prevent toads from entering the road corridor and start wandering between the fences. Alternative mitigation measures to tunnels and fences should always be considered, such as the creation of breeding waters on both sides of the road.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations and approval of N. van den Brink, licensed coordinator animal experiments at Wageningen Environmental Research.

AUTHOR CONTRIBUTIONS

FO and EvdG: study design and data collection; EvdG: data analyses and writing manuscript; FO: reviewing manuscript.

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REVIEW
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A Review of the Factors That Determine Whether Stormwater Ponds Are Ecological Traps And/or High-Quality Breeding Sites for Amphibians

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Stormwater ponds were originally constructed to control the quantity and quality of runoff on urban roads and highways before it was released to the environment. Often, stormwater ponds were designed in a technical feat of civil engineering, with no particular ecological or landscape objective in mind. Nevertheless, they are colonized spontaneously by diverse species, including amphibians. Through an initial review of the scientific literature, the objective of this study was to understand which factors determine whether a pond can be considered as an ecological trap or a valuable breeding site for amphibians. The first step was to question the role of the pond environment as a major factor in its colonization by amphibians, demonstrating that not all ponds are colonized by the same variety of species. The internal factors in the ponds that define them as ecological traps or sustainable breeding sites for amphibians was also considered. After confirming the functional and structural similarity between highway and urban stormwater ponds, 25 publications were compared, with study sites mostly located in Europe and North America, which concern the colonization of stormwater ponds by amphibians in urban or highway areas. Several factors were identified that may affect the ecological viability of these basins: (1) the factors related to the shape of the ponds (inclination of the banks, materials used, etc.,); (2) the biotic factors (aquatic vegetation, presence of predators, etc.,); (3) the abiotic factors (luminosity, water level in the ponds, etc.,); and (4) water pollutants. The low number of publications on this subject, as well as the low variety in the location of study sites, only allow cautious conclusions to be drawn. In particular, stormwater ponds located in highly anthropogenic landscapes can be both ecological traps and suitable habitats for amphibian breeding. This depends on the species that colonize each pond, many internal factors, and the environmental context in which it is embedded. Additional research is therefore needed in other parts of the world—particularly in amphibian biodiversity hotspots—as well as other impact factors such as the effects of different maintenance practices.

Keywords: stormwater ponds, amphibians ecology, transport infrastructures, urban ecology, ecological management

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INTRODUCTION

There is no doubt that there is an increase of the extension of the urbanized land and transport infrastructure. The World Urbanization Prospects report notes that "among 233 countries or areas, just 24 per cent had levels of urbanization greater than 50 per cent in 1950 and only 8 per cent were more than 75 per cent urban. By 2014, 63 per cent of countries were more than half urban and one-third was more than 75 percent urban" (United Nations, 2014). This trend is accompanied by a rise in the number of stormwater ponds in the urban landscape. This has resulted in the creation of a vast network of wetland micro-zones and ecological spaces, which have been quickly colonized by a wide variety of organisms (Scher, 2005), including various amphibian species (Le Viol et al., 2009, 2012; Simon et al., 2009; McCarthy and Lathrop, 2011).

The main role of stormwater ponds is to reduce the environmental impact of water pollution by controlling the quantity and quality of water that is discharged into the receiving environment (Skriabine et al., 2004; Andrews et al., 2015). They are defined in literature as moderately-open surfaces and deep-water systems, initially built to control water runoff and pollution (Scher, 2005; Fayoux and Pelletier, 2009; Le Viol et al., 2009, 2012; Tixier et al., 2011). As Geai et al. (1997) clearly explain, the traditional design of highway ponds is essentially based on technical recommendations from civil engineering, which favors the construction of ponds with regular geometric shapes and varying slope inclination, made of artificial materials (often concrete) that often do not take into account the pond's aesthetic or ecological quality. Two types of stormwater ponds may be distinguished: those located in urban areas and those located on the edge of highways. When the biotic and abiotic characteristics of highway and urban stormwater ponds are compared (see Table in Supplementary Materials), similarities can be found. Although both tend to have a similar average size, the size of stormwater ponds can vary from 173 to 7,000 m^2 for highways ponds (Scher, 2005; Simon et al., 2009; Pohl et al., 2015) and from 49 to 14,784 m^2 for urban ponds (Bishop et al., 2000; Simon et al., 2009; Brand and Snodgrass, 2010; Scheffers and Paszkowski, 2013). Aquatic vegetation generally covers part of the pond water surface, with an average of 55% for highway ponds (Le Viol et al., 2009) and 38% for urban ponds (Bishop et al., 2000; Gledhill et al., 2008; Scheffers and Paszkowski, 2013; Holzer, 2014). Fish are found in 28% of the highway ponds (Le Viol et al., 2009) and 25% of urban ponds (Holzer, 2014). Water features are often similar, with temperatures in temperate regions hovering around 16°C in March (Scher, 2005; Gallagher et al., 2014), a slightly basic pH and a conductivity of around 0.80 mS/cm (e.g., Scher, 2005; Gledhill et al., 2008; Le Viol et al., 2012; Pohl et al., 2015). Whether on highways or in urban areas, a similar proportion of each category of ponds can be found, characterized by the variation of the water level. On highways, Le Viol et al. (2012) found 58% of permanent ponds and 42% of temporary ponds while Scher (2005) found 16% of permanent ponds, 66% of semi-permanent ponds and 18% of temporary ponds. In urban areas, Gallagher et al. (2014) found 36.5% of temporary ponds, 20.6 % of seasonal ponds and 42.8 % of quasi-permanent ponds while Holzer (2014) found 43.5 % of temporary ponds and 56.4% of permanent ponds. Dissolved oxygen levels are quite different. In highway ponds, Pohl et al. (2015) and Scher (2005) found 108% on average. In urban ponds, Gledhill et al. (2008) found 50% but this difference can be due to the fact that only one publication mentions this rate for urban ponds. Finally, in urban ponds, (Scheffers and Paszkowski, 2013; Gallagher et al., 2014; Holzer, 2014) found on average 54 mg/kg of chromium, 29.6 mg/kg of nitrate, 51.3 mg/kg of copper, 212.5 mg/kg of zinc, 0.3 mg/kg of cadmium, 30.1 mg/kg of lead and 662 mg/kg of carbon-hydrogen. There is no data on highway ponds. Although they were not built to host biodiversity, stormwater ponds are colonized by many species, both flora and fauna, common or rare (e.g., Bishop et al., 2000; Ackley and Meylan, 2010; Le Viol et al., 2012; Moore and Hunt, 2012). In some conditions, stormwater ponds biodiversity has been considered equivalent to that of semi-natural wetlands (Hassall and Anderson, 2015).

The 12th session of the Ramsar Convention on Wetlands, held in Uruguay in June 2015, estimated that the global extent of wetlands had declined between 64% and 71% in the twentieth century and that wetland losses and degradation continue worldwide (Gardner et al., 2015). According to Bateman (2014), wetland decline is accompanied by a significant decrease in the global population of amphibians, especially over the past few decades (Blaustein et al., 1994; Houlahan and Findlay, 2003; Dodd, 2010). Because of their biphasic lifestyles, amphibians are subject to both aquatic and terrestrial threats, including habitat loss and degradation (e.g., Berger et al., 1998; Dodd and Cade, 1998; Thomas et al., 2004; Todd et al., 2009; Becker et al., 2010; Bancroft et al., 2011). According to Hayes et al. (2010), death and reproductive failure are the two immediate causes of amphibian decline. Atmospheric change, environmental pollutants, habitat modification, and invasive species are considered as the 4 indirect factors contributing to amphibian decline caused by reproductive failure (Hayes et al., 2010). It is therefore not uncommon to notice the presence of several species of amphibians in urban or highway stormwater ponds. One of the first reactions of the pond managers to this spontaneous colonization was trying to prevent them from entering the ponds, particularly with nets or screens. Few studies have been done to confirm or refute their effectiveness. Another solution to be considered is the possibility to design and maintain these ponds to be viable sites for amphibian reproduction but it is first necessary to determine the features that have an influence on whether the ponds are ecological traps or valuable breeding sites for amphibians.

The concept of ecological trap was first described by Dwernychuk and Boag (1972), but has only been studied in recent years (Battin, 2004). According to Brand and Snodgrass (2010), considering the principle that organisms select high-quality habitats from environmental signals, an ecological trap occurs when environmental clues provide an inaccurate representation of a habitat's suitability for reproduction and survival (Schlaepfer et al., 2002; Battin, 2004; Robertson and Hutto, 2006). A stormwater pond could be defined as an ecological trap if it leads to direct mortality of individuals or if, as a breeding site, reproductive success is not high enough to support a stable or growing population without immigration (Battin, 2004). Due to the proximity of roads that may cause an increase of water pollutants in the pond and also other impacts such as a barrier effect and an increased risk of mortality, highway stormwater ponds do not appear to be suitable breeding sites for amphibians. The presence of water during the breeding period (from spring to early summer) and of vegetation in the ponds may attract amphibians, thus turning these ponds into ecological traps (Schlaepfer et al., 2002; Battin, 2004; Robertson and Hutto, 2006; Brand and Snodgrass, 2010). The purpose of this literature review is to ascertain to which extent stormwater ponds are colonized by amphibians and to identify the factors that would make a stormwater pond an ecological trap vs. a viable breeding site.

METHODS AND MATERIALS

The selection of papers to be evaluated in the study was undertaken using several databases specialized in ecology and geography (Science Direct, Springer, Jstor). Words used in the search were "*stormwater pond*" (1980 results) associated with "*amphibians*" (96 results), "*highways*" (390 results) and "*road*" (890 results). The publications studied cover all types of environmental contexts (agricultural, urban, and forest environments), though most of them are located in developed countries, in particular on the European and North American continents. Though the subject of this study concerns highway stormwater ponds and amphibians, the limited number of publications on this subject (5) resulted in a complementary search through other publications referring to "*urban stormwater ponds*". As highways and urban ponds show high similarities (see Table in Supplementary Materials), 25 publications were selected on both urban and highway ponds: 50% of these publications were from journals of biology, 36% from conservation ecology journals and 14% from environmental science journals, the categories of journals having been determined according to the keywords used. Then, using this bibliographic database from Science Direct, Springer, Jstor as well as a complementary panel of publications on the ecology of amphibians (46), several tables were built to compare the species identified in ponds and the factors influencing—both positively and negatively—the species richness present in the stormwater ponds. There are many factors that can influence the ecological viability of stormwater ponds as a breeding site for amphibians. The effect of 4 categories of factors has been analyzed according to the definitions proposed by Jumeau (2017): (1) the factors related to the design of the ponds (inclination of the banks, materials used, etc.,); (2) the biotic factors (aquatic vegetation, presence of predators, etc.,); (3) the so-called "immediate" factors (luminosity, winds, water level in the ponds, etc.,) and (4) water pollutants. This results in a total of 77 factors. However, some of these factors have not been studied in other research. Therefore, only 37 factors were included in the comparison as they were considered in at least 2 publications.

RESULTS

The Number of Colonizing Species Depends on the Pond's Environment

44 amphibian species were identified colonizing stormwater ponds in the 25 publications included in the analysis (**Table 1**). Three publications on highway ponds referred to 13 amphibian species. 22 publications on urban ponds referred to 37 amphibian species and 17 of these species are cited in at least 2 publications. 6 species were found to be the most common to both urban and highway ponds: the green frog (*Rana clamitans*) and the pickerel frog (*Rana palustris*), which were identified in 5 studies; the American toad (*Bufo americanus*) and the green frog (*R. clamitans*) identified in 6 studies; the spring peeper (*Pseudacris crucifer*) and the wood frog (*Rana sylvatica*), which were identified in 7 studies.

Despite these findings, several articles have reported that species richness depends on the pond's environment and can therefore vary from one site to another. The importance of the landscape matrix for amphibians is regularly examined in the literature, within a radius of up to 500 m around the ponds (e.g., Semlitsch and Bodie, 2003; Simon et al., 2009). The species richness of amphibians is often correlated with a small impervious surface, the proximity to woodlands (Dodd and Cade, 1998; Le Viol et al., 2009, 2012; McCarthy and Lathrop, 2011) and the presence of a dispersal corridor in the case where the breeding site is not directly connected with woodlands (Semlitsch and Bodie, 2003; Ouellet and Leheurteux, 2007; Hamer and McDonnell, 2008; McCarthy and Lathrop, 2011). A terrestrial suitable habitat also provides food and the necessary overwintering sites for amphibians to survive (deMaynadier and Hunter, 1995).

Many studies point out that the density of forest cover has a moderate influence on the presence of amphibians in ponds (Bishop et al., 2000), but this influence varies according to species (e.g., Simon et al., 2009; Birx-Raybuck et al., 2010; Le Viol et al., 2012; Holzer, 2014). As shown by Gallagher et al. (2014), sensitive species such as the wood frog (*R. sylvatica*) occupy only ponds surrounded by a high proportion of forest cover, contrary to more tolerant species such as toads (Scher and Thièry, 2005; Simon et al., 2009). Thus, the literature review shows a correlation between the decrease in forest cover in the surrounding landscape and the decrease in the amphibian species richness identified in the ponds (Le Viol et al., 2009, 2012; Simon et al., 2009). Conversely they demonstrate that species richness and the occurrence of individual species were negatively related to impervious built surface cover (Scher and Thièry, 2005; Simon et al., 2009). The surrounding agricultural matrix is also identified as having a negative influence on species richness, particularly where intensive farming is practiced, probably due to the release of fertilizers and pesticides (Beja and Alcazar, 2003; Le Viol et al., 2012) and/or because it creates a break in the connectivity of the pond and the natural habitat that amphibians depend on (Trenham et al., 2003; Parris, 2006).

Stormwater ponds therefore host a wide variety of amphibian species, but species richness may vary depending on the

TABLE 1 | Species identified in highway and urban stormwater ponds.

Species	Urban Ponds (references)	Highway Ponds (references)
Agile frog (*Rana dalmatina*)		Le Viol et al., 2009, 2012
Alpine newt (*Ichthyosaura alpestris*)		Le Viol et al., 2009, 2012
American Bullfrog (*Lithobates catesbeianus)*	Simon et al., 2009; Birx-Raybuck et al., 2010; McCarthy and Lathrop, 2011; Bateman, 2014	
American Toad (*Bufo americanus*)	Bishop et al., 2000; Massal et al., 2007; Snodgrass et al., 2008; Simon et al., 2009; Brand and Snodgrass, 2010; Bateman, 2014	
Boreal chorus frog (*Pseudacris maculata*)	Scheffers and Paszkowski, 2013	
Common eastern froglet (*Crinia signifera*)	Parris, 2006; Hamer and Parris, 2011	
Common frog (*Rana temporaria*)	Gledhill et al., 2008	Le Viol et al., 2009, 2012
Common toad (*Bufo bufo*)	Gledhill et al., 2008	Le Viol et al., 2009, 2012; Pohl et al., 2015
Cope's gray treefrog (*Hyla chrysoscelis*)	Massal et al., 2007; Simon et al., 2009; Birx-Raybuck et al., 2010; Brand and Snodgrass, 2010	
Eastern Newt (*Notophthalmus viridescens*)	Simon et al., 2009	
Edible frog (*Pelophylax esculentus*)		Le Viol et al., 2009, 2012
Emerald-spotted tree frog (*Litoria peronii*)	Parris, 2006	
European Fire Salamanders (*Salamandra salamandra*)		Le Viol et al., 2009, 2012
Fowler's Toad (*Bufo fowleri*)	Simon et al., 2009; Birx-Raybuck et al., 2010; McCarthy and Lathrop, 2011; Bateman, 2014	
Gray Treefrog (*Hyla versicolor*)	Bishop et al., 2000; Massal et al., 2007; Simon et al., 2009; McCarthy and Lathrop, 2011; Bateman, 2014	
Green frog (*Rana clamitans*)	Bishop et al., 2000; Massal et al., 2007; Simon et al., 2009; Brand and Snodgrass, 2010; McCarthy and Lathrop, 2011; Bateman, 2014;	Scher and Thièry, 2005
Growling grass frog (*Litoria raniformis*)	Parris, 2006	
Haswell's froglet (*Paracrinia haswelli*)	Parris, 2006; Hamer and Parris, 2011	
Long-toad salamander (*Ambystoma macrodactylum*)	Holzer, 2014	Le Viol et al., 2009, 2012
Mediterranean Tree Frog (*Hyla meridionalis*).		Scher and Thièry, 2005; Le Viol et al., 2009, 2012
Natterjack Toad (*Bufo calamita*)		Scher and Thièry, 2005
Northern crested newt (*Triturus cristatus*)	Gledhill et al., 2008	
Northern crocket frog (*Acris crepitans*)	Simon et al., 2009	
Northern Leopard Frog (*Lithobates pipiens*)	Bishop et al., 2000; Bateman, 2014; Gallagher et al., 2014	
Northern red-legged frog (*Rana aurora*)	Holzer, 2014	
Northwestern salamander (*Ambystoma gracile*)	Holzer, 2014	
Pacific chorus frog (*Pseudacris regilla*)	Holzer, 2014	
Palmate newt (*Triturus helveticus*)		Scher and Thièry, 2005; Le Viol et al., 2009, 2012
Parsley frog (*Pelodytes punctatus*)		Scher and Thièry, 2005
Pickerel Frog (*Rana palustris*)	Massal et al., 2007; Simon et al., 2009; Brand and Snodgrass, 2010; McCarthy and Lathrop, 2011; Bateman, 2014	
Pobblebonk (*Limnodynastes dumerilii*)	Parris, 2006	
Rough-skinned newt (*Taricha granulosa*)	Holzer, 2014	
Smooth newt (*Lissotriton vulgaris*)	Gledhill et al., 2008	Le Viol et al., 2009, 2012; Pohl et al., 2015
Southern brown tree frog (*Litoria ewingii*)	Parris, 2006; Hamer and Parris, 2011	
Southern bullfrog (*Lithobates grylio)*	Hamer and Parris, 2011	
Southern leopard frog (*Rana sphenocephala*)	Simon et al., 2009; Birx-Raybuck et al., 2010; McCarthy and Lathrop, 2011	
Spotted marsh frog (*Limnodynastes tasmaniensis)*	Parris, 2006	
Spring Peeper (*Pseudacris crucifer*)	Bishop et al., 2000; Massal et al., 2007; Simon et al., 2009; Birx-Raybuck et al., 2010; Brand and Snodgrass, 2010; McCarthy and Lathrop, 2011; Bateman, 2014	
Striped marsh frog (*Limnodynastes peronii*)	Parris, 2006; Hamer and Parris, 2011	

(Continued)

TABLE 1 | Continued

Species	Urban Ponds (references)	Highway Ponds (references)
Victorian smooth froglet (*Geocrinia victoriana*)	Hamer and Parris, 2011	
Western chorus frog (*Pseudacris triseriata*)	Bishop et al., 2000; Gallagher et al., 2014	
Western tiger salamanders (*Ambystoma mavortium*)	Scheffers and Paszkowski, 2013	
Whistling tree frog (*Litoria verreauxii)*	Parris, 2006; Hamer and Parris, 2011	
Wood frog (*Rana sylvafica*)	Bishop et al., 2000; Massal et al., 2007; Snodgrass et al., 2008; Simon et al., 2009; Brand and Snodgrass, 2010; Scheffers and Paszkowski, 2013; Bateman, 2014	

presence of more or less appropriate amphibian habitats in their surroundings.

Factors That Positively or Negatively Influence the Ecological Viability of Stormwater Ponds as Breeding Sites

The factors that can affect the viability of stormwater ponds as good breeding sites for amphibians cannot be overlooked. Indeed, although they host many amphibians, stormwater ponds are not necessarily favorable habitats for the species that breed there.

Water pollution is one of the most studied factors in the relationship between stormwater ponds and amphibians (**Table 3**). Pollutants such as road salt may have sub-lethal effects on amphibians, which could lead to death in the long term (e.g., Bishop et al., 2000; Sanzo and Hecnar, 2006; Karraker et al., 2008; Snodgrass et al., 2008; Collins and Russell, 2009). However, some authors point out that road salt remains a factor that can slow down the development of the larvae but that it is not decisive in assessing the viability of the ponds as amphibian breeding sites (Scher and Thièry, 2005; Brand et al., 2010; Hassall and Anderson, 2015). Moderate levels of nitrogen in ponds appear to have little or no direct risk on the development of amphibian embryos and larvae identified on site (Mayer et al., 1996; Bishop et al., 2000; Massal et al., 2007). Snodgrass et al. (2008) point out that the impact of pollution on the populations of amphibians present in the ponds studied depends on the tolerance of each species to each of the pollutants. For example, nitrate (NO_3^-) emissions from cars may be an important nutrient for aquatic vegetation (Camargo et al., 2005 in Holzer, 2014). Conversely, high levels of nitrate may have detrimental effects on amphibian larvae due to its toxicity or due to anoxia resulting from eutrophication (Marco et al., 1999; Hatch and Blaustein, 2003; Holzer, 2014). The impact of the nitrate levels also varies positively (Scheffers and Paszkowski, 2013) or negatively (Houlahan and Findlay, 2003) according to the species considered. In fact, stormwater ponds containing moderate nitrate levels may be suitable for the breeding and development of amphibian larvae because it contributes to the development of algae, micro-organisms, and decaying material which amphibian larvae feed on (Duguet et al., 2003; Pohl et al., 2015). Neighboring agricultural areas can also contribute to the development of algae in ponds through the runoff of nitrate-rich fertilizer (Beja and Alcazar, 2003). The odors produced by algae proliferation attract frogs when they're on a reproductive migration (Savage, 1961; Grubb, 1973, 1975; McCarthy and Lathrop, 2011). Algae also nourish amphibian larvae (Bateman, 2014; Holzer, 2014). However, nitrates may also be harmful when present in high concentration, as excessive algae growth can lead to eutrophication (Bishop et al., 2000). In conclusion, agricultural land-use near ponds can have a variable impact, depending on agricultural practices and the sensibility of amphibian species to euthophication (Le Viol et al., 2012).

Some studies point to hydroperiod (**Tables 2**, **3**) as a factor affecting species present on studied sites (Hamer and McDonnell, 2008; Chester and Robson, 2013). Hydroperiod is defined as the time of inundation during which the soil becomes saturated in water, resulting in anoxia (Bonis, 2014). These alternations of flood and wet stages can lead to the coexistence of species with a wide range of tolerance and ecological requirements (Bonis, 2014). The literature has shown that a too short hydroperiod can be harmful to species that have a long developing period, which are unable to reach metamorphosis before the pond dries. Those species die from dehydration (Ostergaard et al., 2008; Brand and Snodgrass, 2010; McCarthy and Lathrop, 2011). Conversely, a too long hydroperiod (i.e., a prolonged duration of submersion) is often associated with the presence of fish, which represent a significant risk of predation for the communities of amphibians present in ponds (e.g., Hamer and Parris, 2011).

A comparison of both **Tables 2**, **3** shows a divergence of opinions on the predation issue. The absence of fish is considered a positive factor in five studies (Beebee, 1996; Petranka et al., 2004; Porej and Hetherington, 2005; Vasconcelos and Calhoun, 2006; Chester and Robson, 2013) and a negative factor in three other studies (Sredl and Collins, 1992; Bishop et al., 2000; Brand and Snodgrass, 2010). The presence of fish has direct and indirect negative impacts on frog larvae (e.g., Porej and Hetherington, 2005; Hamer and Parris, 2011) but some species show resistance to fish predation (Kats et al., 1988; Gunzburger and Travis, 2005; McCarthy and Lathrop, 2011). Brand and Snodgrass (2010) recommend a seasonal hydroperiod, natural or artificial, with a late drainage (i.e., at the end of summer), to increase the suitability of stormwater ponds as amphibian breeding sites. In addition, this practice can be adopted in the maintenance of highway and urban ponds in temperate areas where spring and autumn periods show high levels of rainfall while in summer they have less precipitation.

The pond banks inclination is cited in the literature as a characteristic that can make ponds traps for amphibians

TABLE 2 | Factors identified in the literature review that have a positive effect on the use of stormwater ponds as breeding sites for amphibians.

Factors with positive effects	References of the studies
Ecological connectivity	Prunier et al., 2014
Connectivity with other bodies of water or between ponds	Gledhill et al., 2008; Birx-Raybuck et al., 2010; Chester and Robson, 2013; Hassall and Anderson, 2015
Connectivity with surrounding amphibian populations site	Hamer and McDonnell, 2008
Proximity to terrestrial habitat	Semlitsch, 1998; Guerry and Hunter, 2002; Rubbo and Kiesecker, 2005; Trenham et al., 2005; Van Buskirk, 2005; Babbitt et al., 2006; Rittenhouse and Semlitsch, 2007; Gledhill et al., 2008; Hamer and McDonnell, 2008; Le Viol et al., 2009, 2012; McCarthy and Lathrop, 2011; Chester and Robson, 2013; Holzer, 2014
Possibility of dispersal and/or possibility of colonization of new area	Semlitsch and Bodie, 2003; Ouellet and Leheurteux, 2007; Hamer and McDonnell, 2008; McCarthy and Lathrop, 2011
Proximity to habitat that provides food and wintering site	deMaynadier and Hunter, 1995
Proximity to a wet forest	Baldwin et al., 2006; Holzer, 2014
Appropriate management	Chester and Robson, 2013
Appropriate variations of hydroperiod	Hamer and McDonnell, 2008; Chester and Robson, 2013
Water quality	Hamer and McDonnell, 2008
Low nitrate level	Holzer, 2014
Absence of fish or other predators	Beebee, 1996; Petranka et al., 2004; Porej and Hetherington, 2005; Vasconcelos and Calhoun, 2006; Chester and Robson, 2013
Aquatic vegetation (providing refuge against predators, nesting site, shade and production of oxygen)	Sredl and Collins, 1992; Hamer et al., 2002; Egan and Paton, 2004; Pearl et al., 2005; Skidds et al., 2007; Hamer and McDonnell, 2008; Hamer and Organ, 2008; Hamer and Parris, 2011; Chester and Robson, 2013; Holzer, 2014

(**Table 3**). For example, Parris (2006) registers, on the basis of a predictive model, a decrease of more than 40% in the species richness measured in stormwater ponds due to the presence of a vertical wall. Other factors are mentioned as having an effect on the amphibian presence in stormwater ponds, but do not appear to be conclusive. This is the case for the age of ponds (e.g., Birx-Raybuck et al., 2010; Pohl et al., 2015). Although Birx-Raybuck et al. (2010) identified the presence of amphibians in recent ponds, some species may be slower to colonize new wetlands and are therefore likely to occupy older ponds. The presence of aquatic vegetation within the pond studied is also mentioned as a factor favorable for amphibian development (**Table 2**), without being defined as a determining factor (Brand and Snodgrass, 2010; Hamer and Parris, 2011; Scheffers and Paszkowski, 2013). Similarly, the connectivity of the ponds studied to the surrounding wetlands (**Tables 2**, **3**) is also correlated to the species richness and abundance (Gledhill et al., 2008; Birx-Raybuck et al., 2010; McCarthy and Lathrop, 2011; Hassall and Anderson, 2015).

Certain Negative Factors May be Modified by Stormwater Pond Managers to Make Them Ecologically Viable for Amphibian Breeding

As previously mentioned in the results, some factors influencing the suitability of stormwater ponds as amphibian breeding sites can be modified during pond maintenance operations (e.g., the presence of predators, hydroperiod or pollution accumulation) (e.g., Snodgrass et al., 2008; Birx-Raybuck et al., 2010; Brand and Snodgrass, 2010; Hamer and Parris, 2011). The connectivity between the ponds and suitable natural habitats in the surroundings can be enhanced by vegetation management (Hamer and Parris, 2011; McCarthy and Lathrop, 2011). Similarly, the connectivity of the studied ponds to other wetland habitats (including other stormwater ponds) can be enhanced by vegetation management (Gledhill et al., 2008; Birx-Raybuck et al., 2010; Chester and Robson, 2013; Hassall and Anderson, 2015) in order to support the creation of a dispersal corridor and form a network (Hamer and McDonnell, 2008; Le Viol et al., 2009; Hamer and Parris, 2011). Bateman (2014) suggests that ponds be managed in groups rather than individually to ensure that the habitat requirements of the different species are respected, while improving the species richness on a regional scale.

Finally, other authors Geai et al. (1997); Chang et al. (2011), and Scheffers and Paszkowski (2013) recommend the construction of gently sloping banks, which facilitate the growth of aquatic and semi-aquatic vegetation, as is already the case in many ponds. Chang et al. (2011) recommend that ponds with vegetated banks have slopes $\leqq 45^\circ$, those designed with concrete subtract $\leqq 60^\circ$ and those designed with clay $\leqq 30^\circ$ in order to allow amphibians to climb more easily. In addition to facilitating the entry and exit of amphibians, a structure covered by vegetation provides shelter against predators (Geai et al., 1997; Scheffers and Paszkowski, 2013) and facilitates access for maintenance (Geai et al., 1997).

DISCUSSION

This literature review demonstrates that the viability of stormwater ponds as breeding sites depends largely on

TABLE 3 | Factors identified in the literature review that have a negative effect on the use of stormwater ponds as breeding sites for amphibians.

Factors with negative effects	References of the studies
Lack of connectivity	Ostergaard et al., 2008; Birx-Raybuck et al., 2010
Changes in the hydrological cycle	Hamer and McDonnell, 2008; Brand and Snodgrass, 2010
Long or permanent hydroperiod facilitating fish presence	McCarthy and Lathrop, 2011
Seasonality	Stebbins and Cohen, 1995; Babbitt, 2005
Presence of fish or other predators	Sredl and Collins, 1992; Bishop et al., 2000; Brand and Snodgrass, 2010
Water pollution (affecting survival and reproduction)	Phillips, 1990; Blaustein et al., 1994; McCarthy and Lathrop, 2011
Toxicity of pollutants	Campbell, 1994; Rouse et al., 1999; Gillespie, 2001; Hatch and Blaustein, 2003; Houlahan and Findlay, 2003; Kats and Ferrer, 2003; Massal et al., 2007; Otto et al., 2007; Wik et al., 2008
Oil	Neff et al., 2005
High level of nitrate	Snodgrass et al., 2008
Conductivity and heavy metal	Hatch and Blaustein, 2003; Le Viol et al., 2012
Pesticides and herbicides	Beja and Alcazar, 2003; Le Viol et al., 2012
Salinity	Marsalek and Marsalek, 1997; Bishop et al., 2000; Marsalek, 2003; Karraker et al., 2008; Snodgrass et al., 2008; Denoel et al., 2010; Gallagher et al., 2014
High level of nutrients (causing aquatic eutrophication)	Paul and Meyer, 2001; Johnson et al., 2007
Decrease of algae quantity	Paul and Meyer, 2001
Proximity to urban areas	Hitchings and Beebee, 1997; Gagné and Fahrig, 2007; Hamer and McDonnell, 2008
Woody species too close to the pond (causing shade and decreasing water temperature)	Thurgate and Pechmann, 2007
Human disturbance	Rodríguez-Prieto and Fernández-Juricic, 2005
Artificial light affecting amphibians calls and reproduction cycles	Baker and Richardson, 2006
Noise pollution affecting calls	Sun and Narins, 2005; Bee and Swanson, 2007

characteristics of ponds and their surroundings but also on the ecology of the colonizing species. While water pollution seems to be one of the main characteristics for defining stormwater ponds as ecological traps for amphibians (Bishop et al., 2000; Collins and Russell, 2009; Gallagher et al., 2014), many studies show that its effect varies depending on species and pollution levels, (McCarthy and Lathrop, 2011; Bateman, 2014) even though some pollutants do not directly threaten the development of amphibian embryos and larvae (Massal et al., 2007). We could therefore conclude that stormwater ponds may constitute suitable additional or alternative breeding sites for pollutant-tolerant species (Snodgrass et al., 2008; Holzer, 2014; Pohl et al., 2015). A low level of water pollution, such as a low presence of nitrate, may also be positive for amphibians because it contributes to the development of microorganisms that larvae feed on. However, there is a need to establish which levels of pollution can be tolerated, and by which species.

Thus, for several factors such as pollution levels or hydroperiod, it is difficult to make precise recommendations because of the heterogeneity of evaluation criteria presented in the publications, which limits the comparisons. A good example of this heterogeneity is the characterization of ponds in terms of hydroperiod variations. Stormwater ponds can be divided into two categories: *temporary* or *permanent* (Le Viol et al., 2012; Holzer, 2014); or *seasonal* or *semi-permanent* (Brand and Snodgrass, 2010). However, the classification may be more subtle and may include three categories based on annual observations of ponds. Scher and Thièry (2005) suggest a classification of highway ponds according to whether they are always full (*permanent*), have submerged depths (*semi-permanent*), or have a total drying phase exceeding 1 month (*temporary*). Similarly, Gallagher et al. (2014) define three categories of urban ponds according to the duration of flooding, which can be considered *temporary* (<50% of the time), *seasonal* (50–90% of the time) or *quasi-permanent* (>90% of the time) ponds. Thus, the categories of hydroperiods show a high variation in stormwater ponds (Scher and Thièry, 2005; Brand and Snodgrass, 2010; Le Viol et al., 2012; Gallagher et al., 2014; Holzer, 2014) and is probably the determining factor in the suitability of ponds as habitats for amphibians. Nevertheless, the lack of homogeneity within the hydroperiod classification does not allow the comparison of the results published.

Concerning the surrounding land, the negative influence of the adjacent intensive agricultural areas on the presence of amphibians in stormwater ponds can be explained by a strong tendency of these species to avoid these areas (Joly et al., 2001; Rothermel and Semlitsch, 2002). These agricultural land appear to be obstacles to species dispersal. In addition, they can cause a high concentration of pesticides in the water, which can be lethal for some species (Sparling et al., 2001). On the other hand, the positive influence of the presence of other wetlands near the ponds can be explained by the fact that the size of the regional population is often small. Consequently, the persistence of these populations depends on functional metapopulations composed of a network of different ponds (Semlitsch and Bodie, 2003). Finally, the positive influence of a forest environment can be explained by the fact that many species require habitats covered by natural vegetation where they can find refuge and food, such as forests. The close proximity of the pond to a forest environment leads to a lower cost of dispersal for amphibians during seasonal migration phases (Bonte et al., 2012). Otherwise, most of the data presented in the publications analyzed here concerning the pond environment are studied within a radius of 500 m, considered as the distance of influence of an environment in relation to a pond (Simon et al., 2009). This distance is justified by the fact that dispersal movements may range from several hundred meters to one kilometer (Joly and Grolet, 1996; Denoel, 2005; Kovar et al., 2009). Nevertheless, the area studied should be adapted regarding the dispersal ability of each species.

It is important to underline the low number of publications and their restricted location as a large majority of the articles relate to studies conducted on sites located in Europe and North America. Stormwater ponds are used around the world in a variety of forms and environmental contexts that are currently difficult to evaluate in a review article as the literature does not provide information about functionality, maintenance practices and environment features surrounding the pond. Additional studies are needed, including multi-factorial studies to investigate the influence of the combination of factors listed in this article on amphibian development.

It is also necessary to conduct more targeted studies on the different types of stormwater ponds whose operational objectives and management methods may vary. It might be relevant to include a wider range of factors in the analysis and to make comparative studies between different types of ponds. The difficulty in drawing accurate conclusions also comes from the lack of homogeneity in the few publications concerning this subject as shown with the example of the term "hydroperiod."

Therefore, it is difficult to state on the basis of this review, that stormwater ponds are or are not ecological traps, insofar as this depends on many criteria that vary according to the type of pond, its design, the climate and the land uses in the areas adjacent to the pond's location. The ecology of the species that colonize it is also an important factor. However, it is interesting to note that several authors point out that stormwater ponds could be beneficial breeding sites for amphibian species (Bishop et al., 2000), especially in man-made landscapes where aquatic habitats are increasingly rare (Le Viol et al., 2009, 2012; Gallagher et al., 2014). If so, they could make a substantial contribution toward enhancing local and even regional biodiversity (Gledhill et al., 2008).

It is important to remark that many factors that can affect the sustainability of stormwater ponds to provide quality habitat for amphibians have not been studied. This review of the scientific literature raises the question of whether or not stormwater pond maintenance practices can play a role in the adaptation of the ponds as breeding sites for amphibians (Hamer and Parris, 2011; Gallagher et al., 2014; Hassall and Anderson, 2015).

In **Tables 2**, **3**, pond maintenance is only noted as a potential positive factor and is absent from the negative factors (Chester and Robson, 2013). The different maintenance practices include dredging the pond (i.e., removing the sludge that accumulates at the bottom of the ponds) and clearing vegetation (IFSTTAR-LCPC., 2006; Le Viol et al., 2009). Dredging can have a deadly impact on amphibian populations present in the pond if the activity is carried out during the breeding season. Nevertheless, it can also have other potential benefits because it prevents the pond from being filled by mud and consequently from drying out (Duguet et al., 2003; Ruban et al., 2003). In addition, it prevents the pond from the proliferation of invasive species. The management of stormwater ponds also involves vegetation control in and around the ponds. Although aquatic vegetation plays a positive role in spawning and providing refuges against predators (Duguet et al., 2003), an excessive development can result in eutrophication as well as difficulties in water circulation (Hamer and Parris, 2011). There is a need to find a balance between ecological and technical management in order to support the development of amphibian populations without hindering the functioning of the ponds. Further studies are needed to reconsider pond management and to identify the best practices to reduce the negative factors and to enhance positive ones for amphibian development in stormwater ponds.

This literature review suggests a possible compatibility between a purely technical management and ecological management of ponds that can benefit amphibians while maintaining the functionality of the pond. In addition, this review highlights the importance of a proper vegetation management to link these ponds to a terrestrial habitat, which is necessary for the lifecycle of amphibians. Finally, it seems important to consider the temporality of the process in the analysis of the suitability of stormwater ponds as habitats for amphibians. Whether it be short-term because of a variable hydroperiod, or long-term because of the need to be cleaned regularly to avoid filling, the question remains: Can stormwater ponds be considered temporary wetlands, such as the natural temporary Mediterranean pools mentioned by Babbitt and Tanner (2000); Beja and Alcazar (2003); Jakob et al. (2003); Bagella et al. (2010); Ruhí et al. (2012) or the continental pools mentioned by Lukács et al. (2013)? If so, should these ponds be included in local or regional plans to enhance biodiversity?

However, it is possible to conclude that stormwater ponds located in highly anthropogenic landscapes, as is the case in Europe and North America, can be both ecological traps and suitable habitats for amphibian breeding, depending on a number of factors, including the species that colonize them, pond design, and the environmental context in which they are embedded. Additional studies are therefore needed in other parts of the world, particularly where amphibian biodiversity hotspots are located, but also on possible management and maintenance practices and how to link stormwater ponds to quality terrestrial habitats through the creation of ecological corridors.

AUTHOR CONTRIBUTIONS

LC, CC and PP: Contributed conception and design of the study; LC: Organized the database; LC: Performed the statistical

analysis; LC: Wrote the first draft of the manuscript; LC: Wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00040/full#supplementary-material

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Spillage of Viable Seeds of Oilseed Rape along Transportation Routes: Ecological Risk Assessment and Perspectives on Management Efforts

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Seed spillage during handling and transportation promotes establishment and invasion of feral crops into adjacent semi-natural habitats. This is also the case for oilseed rape (OSR, *Brassica napus*), where seed spillage may lead to establishment of herbicide resistant OSR populations in countries without cultivation of genetically modified OSR. Using data from Austria—where cultivation and import of genetically modified OSR are banned—as a prime example, we demonstrate that ports, oil mills, switchyards, and border railway stations to countries with different electric current systems—where trains have to stop—are the sites of primary concern with respect to seed spillage. Based on the results of the Austrian case study we discuss common measures to limit crop seed spillage which include intensified controls at border railway stations and the mode of seed packing during transportation. We further recommend sufficient cleaning both of goods wagons and of loading areas of trucks and ships as well as an appropriate weed management.

Keywords: oilseed rape, transport, seed spillage, feral crop, invasion, mitigation measures, risk assessment, Austria

Seeds of arable crops are regularly spilled during transport and handling activities. These incidents cause intense management efforts and additional costs (Yoshimura et al., 2006). Moreover, the origin and establishment of feral populations along transportation routes contribute to the uncertainty concerning containment of genetically modified (GM) crops outside fields and could therefore interfere with a successful weed management. Here, we focus on oilseed rape (OSR, *Brassica napus*), a frequently spilled crop (Von der Lippe and Kowarik, 2007) with GM lines already in use, to identify spillage hot-spots due to transportation and handling, allowing us to develop perspectives on common management approaches.

Spillage of OSR seeds has intensively been studied worldwide (e.g., Schafer et al., 2011), which makes OSR a primary model system in this context. We chose Austria as study region because this small country is situated in the center of Europe rendering it a nodal point for traffic and international goods carriage.

OILSEED RAPE AS A MODEL SYSTEM FOR SEED SPILLAGE ALONG TRANSPORT ROUTES AND FOR THE MANAGEMENT OF FERAL CROP PLANTS

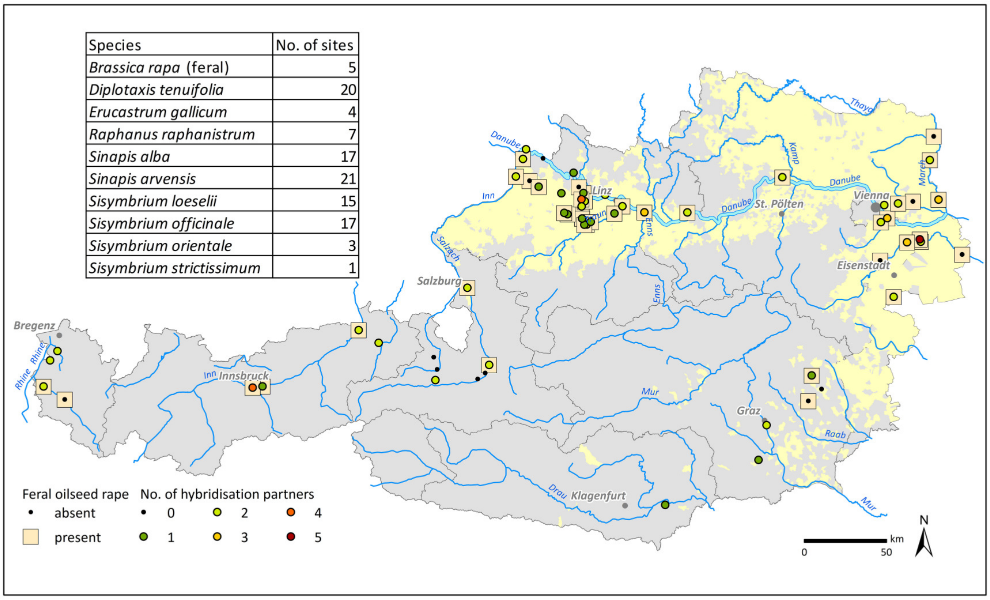
Oilseed rape is a globally widely grown crop of hybrid origin, descending from the parent species cabbage (*B. oleracea*) and turnip (*B. rapa*). It is unknown as a wild plant, but frequently occurs as a feral plant outside cultivation (Pessel et al., 2001). It is mainly pollinated by insects, more rarely by wind, facilitating hybridisation with related species (Chevré et al., 2004), potentially leading to transfer of transgenes into wild populations. For Austria, Pascher and Gollmann (1999) identified more than 20 species as potential hybridisation partners. Oilseed rape seeds are small (1.3–3.1 mm diameter: Rich, 1991) and remain germinable in the soil up to several years (Gruber et al., 2004). At present, GM OSR is widely cultivated in Canada (>90% of OSR cultivation estimated), USA, Chile, and Australia (James, 2015). In the European Union cultivation of GM OSR is currently not authorized, but several OSR lines (e.g., herbicide resistant GT73, MS8, RF3, MS8 × RF3) are admitted for import, processing and feed use.

Feral OSR plants may originate from seed banks at semi-natural sites due to local soil relocation, as a result of epizoochory—diaspores are dispersed *via* adhesion to animals (e.g., wild boars; Schmidt et al., 2004)—or due to seed spillage during transport and handling activities. Transport and handling have been identified as the main reasons for spillage of OSR (Crawley and Brown, 2004; Pivard et al., 2008) and are therefore the focus of the present work. In Central Europe, feral OSR plants are able to reproduce, and populations may persist for several years outside cultivation, especially along transportation routes such as railway lines or roads, as known for France (Pessel et al., 2001; Garnier et al., 2008; Pivard et al., 2008), Germany (Dietz-Pfeilstetter et al., 2006; Menzel, 2006; Elling et al., 2009; Middelhoff et al., 2009; Franzaring et al., 2016), the Netherlands (Tamis and de Jong, 2010), Great Britain (Crawley and Brown, 2004; Squire et al., 2010) and Austria (Pascher et al., 2000, 2006, 2010). In Switzerland, feral glyphosate resistant OSR (GT73) was identified on four of 79 sample sites (Schoenenberger and D'Andrea, 2012), although like in the European Union GT73 is not permitted for cultivation. These four sites were ports and railway stations on the borders to France and Italy. The feral plants probably originated from spillage of contaminated OSR seed from freight trains (Hecht et al., 2014; Schulze et al., 2014, 2015). Spillage of GM OSR seeds along transportation routes was also confirmed in Japan where cultivation of GM OSR is also prohibited (Saji et al., 2005; Kawata et al., 2009; Nishizawa et al., 2009, 2010; Aono et al., 2011; Mizuguti et al., 2011), the United States (in North Dakota 80% of feral OSR proved to be GM: Schafer et al., 2011; Sagers et al., 2012) and Canada (Yoshimura et al., 2006; Knispel et al., 2008; Beckie and Warwick, 2010; Knispel and McLachlan, 2010). Consequently, transportation and handling activities during import of GM OSR are considered the main cause of unintended occurrence and establishment of feral GM OSR in countries without GM OSR cultivation. In these countries, import restrictions have been put into place because introgression of herbicide resistant transgenes may cause problems with weed management and may negatively affect the integrity of genetic resources in wild relatives (Londo et al., 2010).

To illuminate this issue we assess the mid-term to long-term probability of spillage, establishment and distribution of imported viable OSR seeds, using field data collected along transportation routes and at loading and handling sites in Austria, where the import of GM OSR is banned because of ecological concerns (Pascher, 2011, 2012). Austria relies on import of OSR to meet the demands of the Austrian market. Most of the imported OSR seed originates from European countries (currently mainly Hungary, Serbia and Slovakia), smaller amounts are imported from Chile and New Zealand. Data concerning transportation activities of OSR seeds to and within Austria were, however, difficult to obtain or not made available to us. Seeds are imported as bulk mixtures complying with quality standards (e.g., oil content, low content of erucic acid and glucosinolates, absence of GM material) and thus usually without designation of defined OSR varieties (personal communication from managers of warehouses, ports, railway stations). Therefore, the identity of origin of OSR varieties imported to Austria is unknown. Furthermore, since 2012, OSR belongs to the goods category "other products of vegetable origin" and is no longer recorded separately, rendering traceability impossible. Oilseed rape seeds are transported on ship, trains, and trucks. Whereas, available data for transportation *via* ship are rather detailed, those concerning transport *via* railway and trucks are scanty. For instance, the route taken for transport on the road can be freely chosen by the truck-drivers. The biggest fraction of OSR seeds is transported on roads, also at the expense of the rail transport sector, which lost most of its market shares (information provided by the Austrian Federal Railways).

Case Study in Austria

Sixty observation sites in Austria were selected taking transport routes as well as handling and loading sites for OSR into account (a detailed description of the selection protocol is given in Pascher et al., 2016). These sites include predefined hotspots, where OSR seed spillage is expected to occur frequently due to handling activities (6 railway stations along the Austrian borders; 2 switchyards; 6 ports; 3 oil mills that import OSR; 1 processing company) and randomly selected locations where OSR seed spillage is possible (10 railway stations each within and outside OSR cultivation areas; 11 road sections each within and outside OSR cultivation areas). Surveys were carried out in spring and summer 2014 and 2015. At each observation site information such as population size, growing conditions and stage of maturity was recorded. The presence of feral OSR plants was confirmed along relevant transport routes of goods traffic (Pascher et al., 2016). At 44 out of 60 surveyed sites, feral OSR was registered in 2014 and/or in 2015 (**Figure 1**). These also included some sites outside OSR cultivation areas, where feral OSR is expected to have originated from seed spillage of imported OSR rather than from transport of OSR seeds harvested in Austria. Most of the populations were present in both years, indicating their persistence over years. The plants flowered, had already



Species	No. of sites
Brassica rapa (feral)	5
Diplotaxis tenuifolia	20
Erucastrum gallicum	4
Raphanus raphanistrum	7
Sinapis alba	17
Sinapis arvensis	21
Sisymbrium loeselii	15
Sisymbrium officinale	17
Sisymbrium orientale	3
Sisymbrium strictissimum	1

FIGURE 1 | Location of observation sites in Austria. The areas in light yellow indicate the oilseed rape (OSR) cultivation regions. Feral OSR (beige squares) was found at 44 of altogether 60 surveyed sites. Small and larger spots in different colors represent the number of one up to five different species of potential hybridisation partners of OSR registered at each of the 60 observation sites. The number of sites at which each of the 10 species was recorded is listed in the table.

developed viable seeds and exhibited high vitality. We found that the number of OSR plants was consistently higher in areas with cultivation than in those without (Wilcoxon-Mann-Whitney-Test: $p < 0.001$). At several surveyed sites feral OSR occurred in large numbers, e.g., up to 1,500 plants on a 2 km road section in Upper Austria, predominantly in outside curves, or thousands of individuals on the company premises of the largest Austrian OSR oil mill Bunge. Feral OSR was particularly common along railway lines. Among the 10 potential cross-breeding partners of OSR (Pascher et al., 2010) recorded at the 60 observation sites (**Figure 1**), *Sinapis arvensis* (at 21 sites) and *Diplotaxis tenuifolia* (at 20 sites) were found most frequently. In 25 sample sites, two species were registered, in one sample site even up to five potential hybridisation partners were found. The list of registered species in **Figure 1** may be incomplete, however, because at some surveyed sites observation had to focus on feral OSR only due to safety reasons and limited observation possibilities.

Identification of Most Sensitive Links

The most sensitive links in the transportation and processing chain for seed spillage of imported crops were identified to be spillage during transport and loading. Border railway stations, especially those where trains have to stop because of different electric current systems for running trains (e.g., between Italy and Austria), are hotspots for seed dispersal. Seed spillage along tracks may, however, generally increase, as controls of goods wagons (including checks whether the unloading hatches of the wagons were properly closed) are not made at intra-EU borders (i.e., Austrian border to Germany and Italy, and with the progressive extension of the EU, also to Czech Republic, Slovakia, Hungary and Slovenia). This is of particular concern, because Austria is connected to the railway-network of eight neighboring countries and more than 100 megatons of goods are transported here per year. Containment of truck loads is often inadequate, facilitating seeds spillage off the loading area especially in curves. Crop seeds such as oilseed rape are often transported unpacked on open loading areas of trucks and ships and goods carriage. Only occasionally, they are wrapped in sealed bags. Hence, seed spillage of OSR seeds can occur easily. Moreover, especially at ports in storage areas of unpacked grain, birds were observed to feed on the grain and could therefore pose an additional dispersal factor for OSR grain over longer distances (Wedlich et al., 2016). At locations where seeds are loaded and handled in a loose form, continuous seed spillage can be observed which is especially frequent with OSR. Hence, ports, switchyards, and OSR processing facilities and plants, such as oil mills are hotspots for seed spillage. Here, mixtures of OSR varieties as well as imported OSR seeds are handled. Schulze et al. (2014) confirmed GM OSR spillage at such sites in Switzerland. Although import of GM OSR seeds is banned, GM OSR was found in the St. Johann railway station and the Rhine port of Basel. Loading areas of trucks and ships as well as goods wagons are cleaned by the staff themselves. In general, cleaning is mandatory, but the method and the rigor are not defined. So, because of cursory and insufficient cleaning and small OSR seed size considerable amounts of seeds often remain on these transportation areas in spite of the cleaning (Pascher and Dolezel, 2005). Additionally, defect seals of trucks and goods wagons facilitate seed spillage. Additional hotspots for spillage are railway stations where defect trains are repaired. In the railway station of

Innsbruck we registered a large population of feral OSR on the stabling siding which probably originated from seed dispersal of a defect train.

Weed Management and Impurities in Imported Goods

If seeds have already reached the soil, proper weed management of feral plant populations is necessary to prevent persistence of feral plants. All tracks of the surveyed railway stations were sprayed using spraying wagons. The spraying train uses a detection system to recognize weeds along the tracks allowing selective and precise application of herbicides (pers. communication). Thus, it is possible to significantly reduce the amount of the sprayed herbicide. An additional way for seed dispersal is contamination in other goods. Wheat imports from Canada were identified as potential source for contamination with GM OSR seeds (GT73, MS8 × RF3, MS8, and RF3) in the Rhine port of Basel and in processing facilities of two grain mills in Switzerland (Schulze et al., 2015). Imported wheat, a main agricultural goods handled at the Rhine port of Basel, may contain a low level of impurities of GM OSR (impurity in wheat imported from Canada is estimated to be 0.005% at average). Although currently Austria has a high degree of self-supply of wheat (Pascher, 2013), accidental contamination of GM OSR in imported goods needs to be considered in the future.

PERSPECTIVES ON SAFEGUARD MITIGATION OF SEED SPILLAGE ON LONG-TERM BASIS

The establishment of feral crops such as OSR, especially of herbicide resistant lines, may contribute to the loss of biodiversity along transportation routes as well as at infrastructures in two ways. Firstly, feral OSR is able to form large populations, especially in disturbed and semi-natural habitats. Our study supports that some of these large populations establish in areas where no OSR is grown, which likely is due to import activities. Secondly, because of herbicide resistance of GM oilseed rape, these feral plants establish preferentially in habitats where herbicides are applied or that are affected by spraying in adjacent areas. Due to herbicide application selection pressure supports survival of feral glyphosate resistant plants, and this may lead to an introduction of transgenes into related species (Londo et al., 2010) growing in habitats that have to be kept free of vegetation. Consequently, sprayed railway tracks pose an appropriate habitat for the establishment of herbicide resistant OSR as it was shown in Switzerland (Schoenenberger and D'Andrea, 2012). Hence, in order to minimize and mitigate spillage of imported crops such as (GM) OSR, qualified measures need to be defined and implemented. First of all, the mode of seed packing during transportation plays a crucial role. In most cases OSR seeds are transported unpacked facilitating seed spillage. Spillage can be minimized by the use of sealed bags. The loading areas of ships and trucks should be checked regularly for perishing seals, in which case foam can be used to mend the holes. Moreover, at railway borders in Austria transportation facilities need to be checked for appropriate function, e.g., of unloading hatches. Likewise, applying intensified testing of grain cargos imported by ship might allow GM OSR contamination to be identified before unloading, handling and further transportation (Schulze et al., 2014). Cleaning of loading areas of trucks and ships, cargo boards of vehicles and train wagons, and storage areas at ports or oil mills should be intensified and performed carefully. Thereafter, these areas have to be checked for remaining OSR seeds. As seed spillage cannot be fully prevented, weed management has to be applied. Where possible, alternative weed management such as manual removal of plants (as currently already done in the surveyed Austrian ports), mowing, spraying of organic herbicides or grazing by animals is favored. In feral OSR populations spraying with herbicides may be considered to unmask GM OSR individuals, which then can be selectively removed. In contrast, in Switzerland the ports are sprayed and hence, long-term persistence of glyphosate resistant OSR seems to be very likely (Schulze et al., 2014). Weed control along railway lines is necessary to ensure worker safety as well as stability of railway gravel beds. In Austria, to maintain functionality of the beds, the gravel is regularly washed, reducing the amount of plant seeds in the material. Those measures, already performed for reasons of operational safety, would not be affected by the suggested additional weed management. The proposed safeguard measures that we consider necessary will, however, cause additional costs. The main responsibility to limit seed spillage involves the importers and traders, staff who handles and reloads the seeds as well as seed-processors.

To control appearance and persistence of feral crops along transport routes, a monitoring program for imported herbicide resistant crops such as OSR should be implemented in the future. In this context it needs to be considered that documentation of mode of transportation (ship, train, truck), transportation routes as well as loading and handling sites—so called commodity flows—is necessary to identify relevant sites for monitoring in each country and to implement a program taking local specificities into account. Relevant contact addresses (e.g., oil mills, ports) and sources (e.g., Statistik Austria, INVEKOS) for necessary information need to be provided in advance for an immediate update. For us, interviews with the staff of the contacted facilities have been particularly helpful to make domestic and foreign goods' transportation and handling more transparent. If monitoring of, for instance, OSR seed spillage during import activities were to be performed under financial and time constraints, monitoring should focus on reloading and handling sites of OSR in Austria, where the highest genetic diversity within populations of feral OSR plants, probably tracing back to repeated input of different OSR seeds also from abroad, has been found (Pascher et al., 2016). Besides the possibility to unmask herbicide resistant OSR, ecological concerns of the usage of herbicides exist concerning spraying of complementary herbicides, which could facilitate establishment of feral herbicide resistant OSR in case of GM OSR imports. Second in line for surveying are switchyards, transportation roads as well as railway lines leading to loading, handling and processing facilities. Analysing around 2,000 individual plants will provide comprehensive results for a genetic monitoring in small countries such as Austria (Pascher et al., 2016) or Switzerland (Hecht et al., 2014). This sample size is also financially feasible. Since single OSR varieties could not be characterized with eight SSR-markers (Pascher et al., 2016) a larger budget for genetic analyses must be provided to be able to enlarge the marker-set.

Safeguard mitigation efforts on long-term basis are in general needed to prevent establishment and spread of feral GM crops originating from seed spillage along transportation routes. Similar to OSR, the small seeds of cereals are also regularly spilled along roads (Von der Lippe and Kowarik, 2007) or along railway tracks (personal observation) resulting in numerous feral plants. As little documentation concerning cereal seed spillage exists, further detailed research is of utmost importance, in particular, because GM cereal lines have already been developed and could be in use in the near future. In order to succeed with weed management of feral crops along transportation routes, a broad collaboration both in research and practice for interdisciplinary exchange is necessary. Moreover, for a successful approach standards and international guidelines concerning transport of crops—as it would especially be necessary with OSR seeds—have to be harmonized in future.

AUTHOR CONTRIBUTIONS

KP conceived and designed the study and carried out field work. KP and CHR assembled agrarian data, information on transportation routes and import data. CHR carried out sampling and designed the figure. KP and GMS wrote the manuscript. GG commented it. All authors read and approved the final version of the manuscript before submission.

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Seed Dispersal Distances by Ants Increase in Response to Anthropogenic Disturbances in Australian Roadside Environments

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Ants provide a common dispersal vector for a variety of plants in many environments through a process known as myrmecochory. The efficacy of this dispersal mechanism can largely determine the ability of species to track changes in habitat availability caused by ongoing land-use and associated disturbances, and can be critical for population gene flow and persistence. Field studies were conducted in a typical fragmented agricultural landscape in southern NSW, Australia, to investigate the extent to which dispersal services by ants are influenced by anthropogenic disturbances associated with roadwork activities (i.e., soil disturbance as the result of grading of roads). Observational experiments were performed in road segments that were divided into disturbed and non-disturbed zones, where *Acacia pycnantha* seeds were offered at multiple bait stations and monitored. For combined species, the mean dispersal distance recorded in the disturbed zone (12.2 m) was almost double that recorded in the non-disturbed zone (5.4 m) for all roadside sites. Our findings show that myrmecochory is an unevenly diffuse mutualism, where few ant species contributed to much of the dispersal of seeds. *Iridomyrmex purpureus* was responsible for all seed dispersal distances >17 m, where a maximum of 120 m in disturbed, vs. 69 m in non-disturbed zones, was recorded. *Rhytidoponera metallica* and *Melophorus bruneus* were important seed dispersers in non-disturbed and disturbed zones, respectively. In general, large bodied ants tended to move more seeds to longer distances in disturbed zones, as opposed to non-disturbed zones, where smaller bodied species carried out a greater percentage of short distance dispersals (<1 m). We also recorded secondary dispersal events from nests by *I. purpureus*, a phenomenon previously not quantified. Infrequent, long distance dispersal to suitable sites may be highly important for seedling recruitment in disturbed or modified habitats in otherwise highly fragmented rural environments.

Keywords: acacia, habitat connectivity, myrmecochory, road ecology, soil disturbance

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INTRODUCTION

For many plants, seed dispersal mechanisms following the end of the reproductive cycle are critical for the establishment of future offspring (Wang and Smith, 2002; Vander Wall and Longland, 2004). Plants use various strategies to disperse their propagules into new habitats, such as by wind, water, vertebrate or ants (van der Pijl, 1982; Nathan and Muller-Landau, 2000). Dispersal distances can range from < 1 m (i.e., ants) to > 100 km (i.e., wind) (Corlett, 2009; Thomson et al., 2010).

However, the limited dispersal distances provided by some dispersal agents (i.e., ballistic and/or ant-dispersal) may restrain the extent to which plants may colonize new habitats. As a result, recruitment limitations can occur, particularly in habitats subjected to frequent disturbance activities (i.e., natural and/or anthropogenic). This issue can affect population growth and persistence, unless other forms of infrequent long dispersal events occur (Cain et al., 2000; Nathan et al., 2008).

Seed dispersal by ants (*myrmecochory)* is a globally significant driver of plant diversity and population dynamics (Lengyel et al., 2010). Myrmecochorous plants dominate communities in many habitats, in terms of both species richness and abundance (Berg, 1975; Beattie, 1985; Lengyel et al., 2009). There are numerous ant species that remove and transport seeds, however most dispersal events are performed by a few key removers (e.g., Andersen, 1988; Gove et al., 2007; Zelikova and Breed, 2008). Ants generally disperse seeds only short distances to their nests where they remove the elaiosome to feed to their larvae (Culver and Beattie, 1978; Hughes and Westoby, 1992; Rowles and O'Dowd, 2009; Gómez and Espadaler, 2013). However, infrequent long distance dispersal events can also occur by ants (Andersen, 1988). Maximum recorded seed dispersal distances are 77 m (Davidson and Morton, 1981) and 180 m (Whitney, 2002); both recorded in Australia.

The average dispersal distance, and the nature of the seed dispersal curve, play a crucial role in the rate of colonization of propagules to new sites (Andersen, 1988; Portnoy and Willson, 1993). Recent studies have also highlighted that myrmecochory can be a multiphase process, where after an initial transport to a nest, seeds may then be discarded away from the nest via a secondary dispersal process (Beaumont et al., 2012). Ants may remove discarded seeds to nearby rubbish heaps (Berg, 1975) or be relocated further distances away from nest entrances (Hughes and Westoby, 1992; Lubertazzi et al., 2010; Canner et al., 2012). On most occasions, the elaiosome of the discarded seed is removed (Hughes and Westoby, 1992; Canner et al., 2012), which assists with breaking seed dormancy and influence subsequent germination success of species (Pacini, 1990; Lobstein and Rockwood, 1993).

Infrequent long-distance dispersal events by ants, and seed discarding behavior from ant nests, can have various implications for the fate and distribution of seeds. The extent of this dispersal service is strongly tied to the composition and behavior of the ant species involved, and influenced by prevailing disturbance regimes (e.g., Beaumont et al., 2012). Disturbances can lead to increased dispersal distances, possibly due to simplification of the foraging landscape for ants (Parr et al., 2007). In many habitats, increased anthropogenic activity poses a threat for many species, where understanding the factors that influence ant-plant interactions, such as seed dispersal into new environments, is critical for conservation management (Thomson et al., 2010; Sorrells and Warren, 2011).

We investigated seed dispersal processes by ants in relation to soil disturbances. We undertook investigations in roadside environments in southern NSW, Australia, which provide refuge for endangered woodland communities and associated species (Benson, 1991; Schabel and Eldridge, 2001; Spooner and Lunt, 2004). These novel environments are maintained by anthropogenic activities, such as grading of the road surface and adjacent boundary, which affect existing vegetation along roads, by removing plant biomass and creating a bare soil surface. This process can influence seed dispersal, recruitment and overall plant persistence, depending on their life-history traits (Lugo and Gucinski, 2000; Gelbard and Belnap, 2003; Spooner, 2005). The main objectives of the study were: (i) to investigate the influence of soil disturbance on seed dispersal distances, (ii) identify the relative contribution of individual ant species to this process, and (iii) determine the extent of potential secondary seed dispersal performed by ants.

METHODS

Study Area and Sites

This study is part of a larger research project investigating seed dispersal processes by ants in roadsides and the detailed description of the study area and site selection can be found in a preceding paper (Palfi et al., 2017). In summary, field work was carried out in the Lockhart Shire, a rural local government area located in southern NSW, Australia. The region has a cool temperate climate, with mean annual rainfall ranging from 450 to 600 mm, and altitude ranging from 200 to 450 m. Topography consists of low undulating hills and flat riverine plains, with sporadic granite and porphyry outcrops (Lockhart Shire Council, 2013). Much of the area is arable farmland dominated by cropping and grazing farm systems, subdivided by a network of minor and major roads. As much of the landscape is cleared or highly fragmented, these roads often harbor the last vestiges of "intact" remnant woodlands, grasslands, and other ecosystems (Lunt and Bennett, 2000).

The Lockhart study area contains a large network of roads (~1,600 km), where minor roads represent almost half of the total road network (750 km; Spooner et al., 2004). Minor roads are maintained by human soil disturbance regimes which although deleterious to many plants, can be advantageous to others, depending on their life history and dispersal traits (Lugo and Gucinski, 2000; Gelbard and Belnap, 2003; Spooner, 2005). In previous studies, historical roadwork activities were shown to facilitate the recruitment and persistence of roadside plant populations (Spooner et al., 2004), and promote some *Acacia* species (Spooner, 2005). *Acacias* are important and widespread myrmecochorous genera in Australia and elsewhere (Berg, 1975). As such, roadside environments provide an ideal context to study the impact of soil disturbance on mutualistic interactions such as myrmecochory. The research was confined to this area to ensure that a consistent approach to local government road management was applied across the study area.

A stratified random sampling approach was used to select 24 roadside sites located in the Lockhart Shire council area, southern NSW, where common *Acacia* populations were known to occur (Bull, 1997; Spooner, 2005). Each roadside site can be subdivided into two parts: (1) the disturbed zone adjacent to the road surface (1–5 m from the road edge), which is frequently disturbed (1–2 times per year) by mechanical soil grading operations to clear all above ground vegetation and top 1 cm of soil, adjacent to the road drainage line (hereafter the disturbed zone); and (2) the undisturbed road verge beyond the disturbed zone where intact vegetation occurs, where no direct effects of grading were visible (i.e., hereafter referred to as the non-disturbed zone). Furthermore, as roadside environments occur at varying widths, which in turn influence habitat and disturbance conditions (Spooner and Lunt, 2004), sites were stratified into two groups: narrow (3–14 m) or wide (15–60 m) roadsides (for disturbed and non-disturbed zones combined).

Recording Seed Dispersal Distances

To assess ant dispersal distances, "Cafeteria" style experiments were carried out. Seed depots of ∼9 × 9 cm in size were placed at 5 m intervals along two 25 m transects, located parallel to each other in both the disturbed and non-disturbed zones of the selected roadside site. These transects were separated longitudinally by a gap width of 10–20 m (minimum 10 m in case of narrow road verges). At each depot, 10 seeds of *Acacia pycnantha* Benth. were placed on the ground. Seeds were collected prior to commencing the study during the seed ripening period in 2013 (November-December). The research was carried out during the summer months of December 2013 to March 2014, where two observational periods were appointed: the morning session consisted from 8:00 to 12:00, and the afternoon session from 16:00 to 20:00, time periods comprising highest ant activities.

A seed dispersal event was considered when ants removed seeds at least 5 cm away from the edge of the seed depot, and subsequently, these ants were followed until a destination point was reached. Dispersal distances were measured from the respective seed depot to a given nest (or drop point) using a 50 m tape measure. The fate of dropped seeds (if they were further removed or not) was not monitored, however the dispersal distance of such seeds was also recorded. As *Iridomyrmex purpureus* were common and dispersed seeds often great distances, we did not directly measure every dispersal event performed by this species to its termination point. Rather, the first *I. purpureus* individual transporting a seed from the bait station was followed until it reached a particular nest. *I. purpureus* nests are large and conspicuous, where individual nests are normally situated sufficiently far from each other. Following individuals heading toward the same nest site were then only monitored for ∼5 m from the bait station, until it could be assumed it would complete the journey to this nest (and this distance recorded). Previous trial experiments proved that a large percent (90%) of *I. purpureus* individuals that carried the offered seeds beyond 5 m of the seed bait stations would reach their nests without dropping them. This method was developed in order to maximize the number of observations of ants at the bait stations during the survey period (4 h per session).

All other dispersal events by other ant species were monitored to their final destination, and the distance recorded. If individual ants could not be identified during field work, they were followed until a drop point or ant nest and were captured and placed into vials filled with 70% ethanol for later identification. Collected individuals were later identified in lab condition using field guides or expert advice (Andersen pers. comm. 2014).

Secondary Dispersal Events—Relocation Distances from Ant Nest Entrances

To investigate secondary seed dispersal events, we focussed on *Iridomyrmex purpureus* nests, which were abundant in the sampled roadsides. We randomly selected a total of 34 independent *I. purpureus* nests (disturbed $n = 14$; non-disturbed $n = 20$) at the combined roadside sites. *Acacia pycnantha* seeds were again used to perform these observations, which were collected during the ripening period of 2014 November (prior to the relocation experiment in December 2014). Observations were conducted in the morning period between 9.00 and 12.00 h when most ant activity occurs. As *I. purpureus* builds large and conspicuous nests which have numerous ant entrances, we randomly placed a total of 15 individual seeds/nests ∼5 cm from different nest entrances. Elaiosomes were previously detached of seeds to stimulate seed manipulation behavior of ants that often induces seed discarding behavior from ant nests (e.g., Martins et al., 2006). Seeds were placed individually to a random entrance and if an ant displaced the seed the distance was recorded.

Data Analyses

To compare the mean dispersal distances between disturbed and non-disturbed zones and roadside width we used mixed models (factorial ANOVA) where zone and width were entered as fixed factors, and sites as random factor. To ensure equal weights to each station rather than number of seeds, we first calculated the average dispersal distance for each depot then calculated the average for each zone and site to get the site average distance. Data was log-transformed if the assumption of normality was not met. As *I. purpureus* contributed to all dispersal distances >17 m, we used the same statistical configuration to test for differences in mean dispersal distance with data for *I. purpureus* omitted.

Differences in dispersal distances between zones and widths were investigated for ant species with sufficient dispersal measurements, or more precisely when a species was present in both zones of a site (at least 10 sites). Due to the paired nature of the study design, sites were not included unless dispersal distances were recorded in both zones. Paired T-test or Wilcoxon Signed Rank test was used if the data were normally or not normally distributed, respectively. Descriptive statistics were used to describe differences in mean dispersal distances achieved by respective species in differing zones and roadside widths. A frequency histogram of dispersal distances was constructed to generate a dispersal curve, by calculating the relative frequencies of each zone within each site, then a single composite histogram was aggregated by giving equal weight to each site. By doing this we were able to take site variability into account where the number of dispersal events greatly varied due to the patchy nature of ant activities (Nelson, 2014). The Kolmogorov-Smirnov test was used to test whether the two distributions differed between the zones.

In order to ascertain differences in secondary dispersal distances by *I. purpureus* between disturbed and non-disturbed zones, we calculated the average relocation distance for each *I. purpureus* nest and used a paired Student's t-test with Satterthwaite's correction to compare relocation distances between disturbed and non-disturbed zones. All statistical tests were carried out using R statistical software (R Development Core Team, 2013).

RESULTS

Dispersal Distances in Relation to Soil Disturbance

Overall, mean seed dispersal distances were significantly influenced by soil disturbance (zone) ($F = 4.9$, $df = 1.22$, $p = 0.03$) and roadside width ($F = 6.1$, $df = 1.22$, $p = 0.02$) (**Figure 1**). For combined species, the mean dispersal distance recorded in the disturbed zone was almost double that recorded in the non-disturbed zone (12.2 m ± 3.71 SE vs. 5.4 m ± 1.10 SE, respectively) for all roadside widths combined, while wide roadsides had longer dispersal distances than narrow roadsides. Nevertheless, narrow roadsides had both the longest (14.5 m ± 7.2 SE) and shortest (2.9 m ± 0.81 SE) mean dispersal distances in the disturbed and non-disturbed zones, respectively, while there was no statistical difference in dispersal distances between disturbed and non-disturbed zones in wide roadsides (**Figure 1**).

The main seed dispersing ant genera observed were *Iridomyrmex* (43% of total dispersal events), *Rhytidoponera* (37%), and *Melophorus* (10%) (**Table 1**). Overall, 19 ant species contributed to the dispersal of seeds in roadside environments, with *I. purpureus* and *R. metallica* dominating the seed dispersal activities with 31 and 28%, respectively (data combined across sites and zones). When mean dispersal distances for combined species were re-analyzed by omitting *I. purpureus*, there were no significant differences in mean distances between road disturbance zone or road verge width ($F = 2.33$, $df = 1.22$; $p > 0.05$).

There were no significant differences in the mean dispersal distances of individual species between disturbed and non-disturbed zones, roadsides of different width or the interaction effect ($p > 0.05$) However, there were trends in mean dispersal distances of species according to either disturbance zone and/or roadside width (**Figures 2**, **3**, **Table 1**). *Iridomyrmex purpureus* dispersed the most seeds over the greatest distances in the disturbed zone (mean = 25.1 m, maximum = 120 m; **Table 1**). Their average dispersal distances were considerably greater than any other species' (**Figure 2** vs. **Figure 3**), and reflect the overall pattern for combined species, where the mean seed dispersal distance was greatest in disturbed zone of roadsides (**Figure 3**).

The mean dispersal distance for *Rhytidoponera cristata* for combined sites was 4.40 m ± 0.64 SE, and was greater in disturbed zones for both wide and narrow roadsides (**Figure 2**, **Table 1**). In contrast, the mean dispersal distance for *Melophorus bruneus* was longer in the non-disturbed zone of both wide and narrow roadsides (**Figure 2**). *Rhytidoponera metallica* also followed this pattern for wide roadsides. Mean dispersal distances for *Iridomyrmex rufoniger* were much lower than any other species' (mean 0.60 ± 0.09 m). Some "other" species showed slight preferences for disturbance zone and/or roadside width, where the long mean dispersal distance in the disturbed zone of narrow roadsides was due to activities of *Camponotus* species (**Figure 2**).

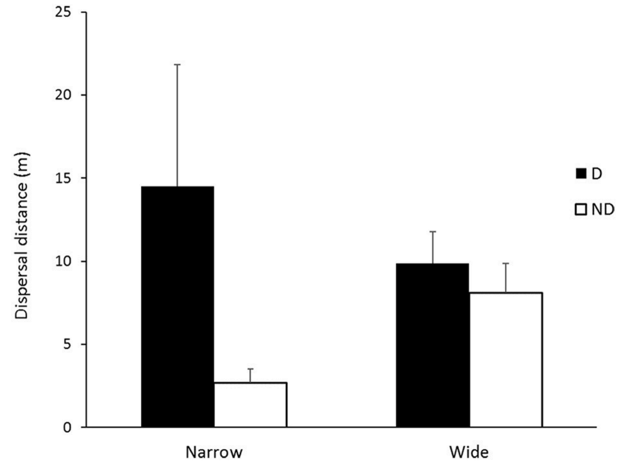

FIGURE 1 | Mean dispersal distances (m) carried out by seed-dispersing ant species in disturbed (D) and non-disturbed (ND) zones in narrow ($n = 579$ in D, $n = 376$ in ND) and wide roadsides ($n = 442$ in D, $n = 553$ in ND) in the Lockhart Shire, southern NSW. Error bars represent standard error of the mean.

Frequency of Seed Dispersal Events

A high frequency (64%) of recorded seed dispersal events was <3 m, where a further 20% of dispersals were carried out at distances ranging 10–40 m (**Figure 4**). The tail end of the dispersal curve shows small frequencies of long distance dispersal events up to 120 m in the disturbed zone. In contrast, the maximum dispersal distance recorded in the non-disturbed zone was 70 m (**Figure 4**). The relative high frequency of seed dispersal distances <3 m was generally similar in both disturbed and non-disturbed zones, however dispersal events >5 m tended to occur more frequently in the disturbed zone of roadside environments (**Figure 4**). Nevertheless, there were no significant differences in the frequency of seed dispersal events between the zones or roadside widths ($p > 0.05$). The maximum distances recorded in this study were by *I. purpureus*: 69 m in non-disturbed and 120 m in disturbed zones.

Secondary Dispersal Distances from Nests

There was a significant difference in the secondary dispersal distances *I. purpureus* carried out between disturbed and non-disturbed zones ($t = 4.12$, $df = 20.73$, $p < 0.001$; **Figure 5**).

DISCUSSION

The ant genera responsible for most seed dispersals were *Iridomyrmex*, *Rhytidoponera* and *Melophorus*. Other studies have also found that these genera are the most important dispersers involved in myrmecochory throughout Australia (Berg, 1975; Andersen and Morrison, 1998; Beaumont et al., 2012). In this study, a total of 18 species were recorded dispersing seeds, with *Iridomyrmex purpureus* and *Rhytidoponera metallica*

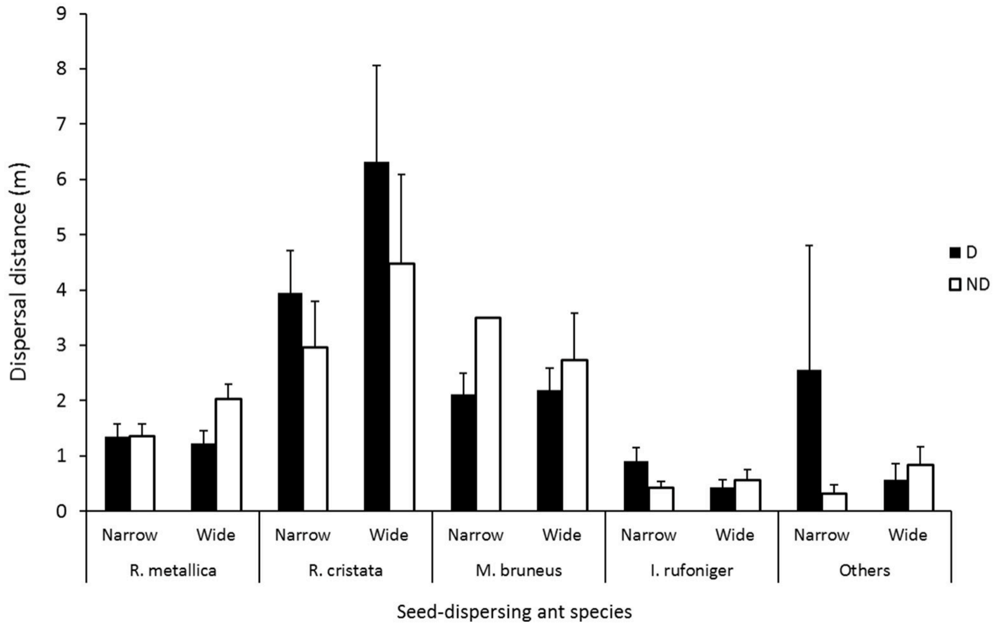

FIGURE 2 | Mean dispersal distances for individual ant species (*I. purpureus* excluded) in disturbed (D; $n = 669$) and non-disturbed (ND; $n = 677$) zones in narrow and wide roadsides. Others = all other remaining species combined (see **Table 1**). Error bars represent standard error of the mean.

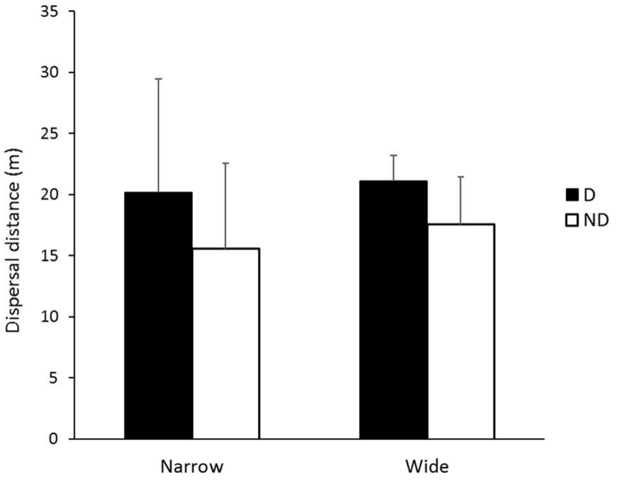

FIGURE 3 | Mean dispersal distances (m) for *Iridomyrmex purpureus* in disturbed (D; $n = 352$) and non-disturbed (ND; $n = 252$) zones in narrow and wide roadside environments. Error bars represent standard error of the mean.

being associated with the most seed dispersals (31 and 28% respectively), the former with maximum dispersal distances. *Melophorus bruneus* carried out a substantial amount of seed dispersal events as well (10%). *Pheidole* is also a well-known seed taking genus (Beaumont et al., 2012), however it was not a prominent disperser in our study. In general, our findings are consistent with the assertion that myrmecochory is an unevenly diffuse mutualism (Gove et al., 2007), that is, few ant species are responsible for the majority of seed dispersal.

Soil disturbance had a significant effect on seed dispersal distances for combined species, where mean distances were greater in disturbed as compared to non-disturbed zones, and the difference was more pronounced in narrow roadsides. This finding can be largely explained by results for *I. purpureus* (see below) which strongly influenced our overall findings, as with data for *I. purpureus* excluded, mean dispersal distances resemble those of other myrmecochorous studies (as reviewed by Gómez and Espadaler, 2013). In a recent review of myrmecochory, Gómez and Espadaler (2013) found a global mean ant dispersal distance of 1.99 m, with greater mean dispersal distances in the Southern Hemisphere (3.71 m). We recorded dispersal distances much greater than this average (mean 5.4 m in non-disturbed, 12.2 m in disturbed zones with data for *I. purpureus* included) in roadside environments.

I. purpureus was largely responsible for all seed dispersal distances longer than 17 m (mean = 25.1 m, maximum = 120 m; **Table 1**), with longer distances in the disturbed zone, which greatly influenced overall results. Such distances have been recorded in similar studies, but in more arid environments. Whitney (2002) recorded exceptionally long distances (180 m) based on direct observations; and more recently, Pascov et al. (2015) recorded a distance of 417 m using microsatellite markers and parentage assignments for seeds found in ant nest middens. Both studies included measurements on species from the *Iridomyrmex* genus in arid environments. This is the first study providing distances to such a degree outside of the Australian arid zone, which suggests that infrequent long dispersal distances may not necessarily be restricted to this environment.

The composition of ant assemblages at any given habitat is known to influence seed dispersal services by ants (Zelikova and

TABLE 1 | Number of seed dispersal events, number of sites ($n = 24$) each species occurred at, and mean and maximum dispersal distances, recorded for ant species in disturbed and non-disturbed roadside sites in southern NSW, Lockhart Shire.

Ant species	**Disturbed zone**			**Non-disturbed zone**		
	Number of dispersal events (No. of sites)	**Mean distance (m)**	**Maximum distance (m)**	**Number of dispersal events (No. of sites)**	**Mean distance (m)**	**Maximum distance (m)**
Iridomyrmex purpureus	352 (19)	25.1	120	252 (17)	16.9	68.6
Iridomyrmex rufoniger	157 (13)	0.4	4	86 (12)	0.5	1.6
Rhytidoponera cristata	57 (13)	5	13.6	47 (12)	3.7	16.5
Rhytidoponera sp. A *(convexa* group)	18 (4)	1.7	3.2	58 (8)	3.1	7.4
Rhytidoponera metallica	266 (18)	1.4	14	311 (24)	1.6	7.9
Melophorus bruneus	132 (15)	2.1	14.7	66 (9)	2.8	6
Camonotus sp. 1	1 (1)	1	1	9 (1)	6.2	14.2
Camponotus sp. A *(claripes* group)				1(1)	1.2	1.2
Camponotus obniger	4 (1)	1.5	3.1	2 (2)	8.6	11.2
Crematogaster sp. A	12 (1)	0.1	0.3	19 (2)	0.1	0.1
Melophorus sp. B (aeneovirens group)	5 (4)	4	14.7	3 (2)	4	6
Meranoplus sp. A (group D)				1 (1)	0.1	0.1
Monomorium sp. B *(rothsteini* group)	19 (3)	0.5	1.5	8 (3)	0.41	1.5
Monomorium sp. A *(sordidum* group)	14 (3)	0.1	0.4	8 (3)	0.1	0.1
Notoncus ectatommoides	2 (1)	0.2	0.3	12 (3)	0.1	0.35
Pheidole sp. A	11 (7)	0.2	0.6	33 (9)	0.1	0.5
Pheidole sp. B				8 (2)	0.1	0.1
Camponotus aeneopilosus	2 (2)	11.5	13.8	4 (3)	0.6	2.5
Total	1,051	12.2	120	929	5.4	68.6

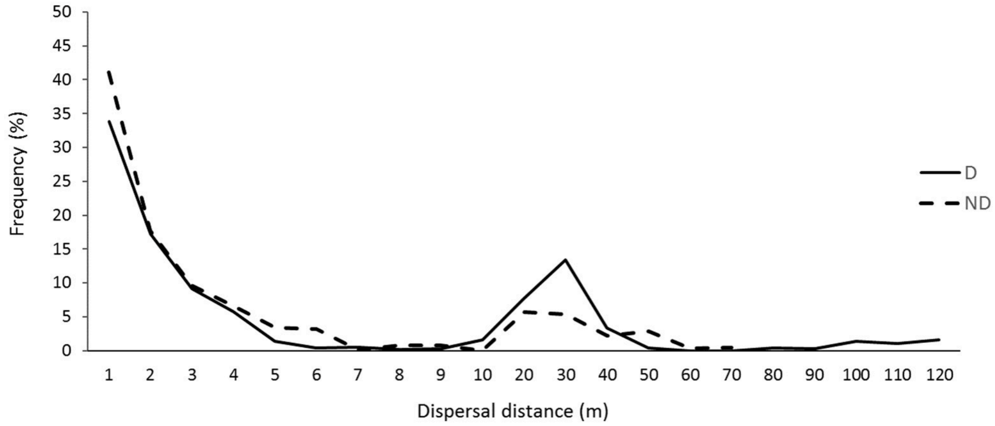

FIGURE 4 | Overall seed dispersal curve across all seed-dispersing ants. Seed dispersal distances are grouped in distance categories (m) in disturbed (D; $n = 1{,}021$) and non-disturbed (ND; $n = 929$) zones of roadside environments in the Lockhart Shire, southern NSW.

Breed, 2008; Beaumont et al., 2013). A previous study along roadsides found a largely similar composition of seed-dispersing ant species between disturbed and non-disturbed zones (Palfi et al., 2017), which can largely explain the lack of significant differences in mean dispersal distances for individual species between zones (**Figure 2**). Furthermore, the patchy nature of ant activities during the experiment resulted in the exclusion of various species from statistical analyses, therefore forming generalizations in terms of soil disturbance effects on individual species is difficult. Parr et al. (2007) also found no effects of burning on mean dispersal distances at the species level; only in the case of an *Iridomyrmex* species. Likewise, Andersen and Morrison (1998) found that *Iridomyrmex* contributed to overall longer mean and maximum distances in relation to disturbance from mining activities.

We found that a somewhat greater percentage of short distance dispersals (<1 m) occurred in non-disturbed vs. disturbed zones, though this difference was not significant

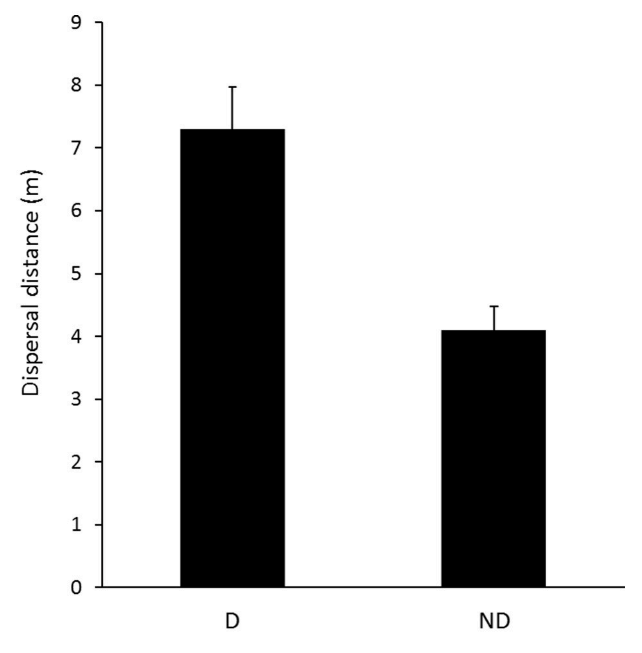

FIGURE 5 | Mean secondary dispersal distances performed by *I. purpureus* colonies between disturbed (D; $n = 111$) and non-disturbed (ND; $n = 169$) zones in the Lockhart Shire. Error bars represent standard error of the mean.

(**Figure 4**), where the activities of *R. metallica* were primarily important. Despite mean dispersal distances of *R. metallica* being generally short (1.5 m), this species is considered as a keystone seed disperser throughout Australia (Gove et al., 2007), pointing to other factors than dispersal distance as important features of the seed dispersal mutualism (i.e., rate of seed removal, burial of seeds; Hughes and Westoby, 1992; Lubertazzi et al., 2010; Palfi, unpubl. data). Other smaller bodied species (*Monomorium* and *Pheidole*) also contributed to the high frequencies of short distance dispersals in both zones. Severely disturbed sites are often coupled with low dispersal distances due to the predominance of small bodied ant species (Pudlo et al., 1980; Andersen and Morrison, 1998; Leal et al., 2013).

Body size has been demonstrated to be a good proxy for assessing likely dispersal distances a species may be able to perform (Ness et al., 2004), and so does their foraging behavior in search of food resources (e.g., Lubertazzi et al., 2010). Therefore, dispersal distance is largely a function of disperser identity (Andersen, 1988; Gove et al., 2007). Given the predominance of large bodied species we recorded, particularly in disturbed zones, and commensurate large distances they dispersed seeds, suggests that the soil disturbance regime imposed in our study is infrequent, or an intensity of which these ants can still effectively nest and forage within.

I. purpureus Activity in Roadside Environments

The distribution and density of ant nests can greatly determine the shape of the dispersal curve at any particular site (Andersen, 1988). The overall pattern of the seed dispersal curve with a long tail of infrequent but exceptionally long dispersal distances, suggests low nest densities (Green, 1983). Under such circumstances, the foraging effectiveness of dispersers becomes very important for persistence of plant communities in such environments.

Where *I. purpureus* were present, nests were discovered in both the disturbed and non-disturbed zones. Seed dispersal distances by *I. purpureus* were strongly influenced by the spatial location of nests, as individual *I. purpureus* foragers returned to the same nest, or on few occasions to multiple nests (Palfi, pers. obs.; van Wilgenburg and Elgar, 2007). This assertion is supported by our seed dispersal distance data (**Figure 4**), where high dispersal frequencies at 30–40 m reflect the observed nest distribution patterns. Second, field observations revealed that *I. purpureus* used the graded soil surface as a "runway" to access resources at great distances in both the disturbed and non-disturbed areas.

Complex vegetation structure is known to reduce the foraging speed and discovery of new resources by ants (Gibb and Parr, 2010), therefore this form of soil disturbance appears to confer advantages for *Iridomyrmex* to access resources in roadside habitats. Undoubtedly, open areas, such as those prevalent in roadsides, may confer additional advantages to *I. purpureus* in terms of suitable warmth, and open conditions for nesting (Greenslade, 1976). It has been suggested that the simplified and obstacle-free habitat conditions which exist post-disturbance, especially from an ant point of view, provide conditions commensurate for effective seed dispersal, especially by larger bodied ants (Davidson and Morton, 1981; Parr et al., 2007).

The secondary seed dispersal activities we recorded for *I. purpureus* may provide additional services for plants, providing the seed remains viable after handling by ants. Whitney (2002) analyzed the density of discarded seeds from *Iridomyrmex* nests (sometimes up to 25 m), and recorded a high seed viability ratio (40%). Furthermore, middens of *Iridomyrmex* nests were reported to provide environmental conditions suitable for seedling growth, and facilitate range expansion of plant species in northern Australia (Bebawi and Campbell, 2004). Other species can also discard seeds from their nests (Beaumont et al., 2013), sometimes with the elaiosomes remaining intact on discarded seeds. The potential benefits of multi stages dispersal processes may be particularly important for plants existing in fragmented environments where the maintenance of habitat connectivity may be crucial for overall population persistence.

CONCLUSION

As many landscapes have been cleared for agricultural purposes in Australia and elsewhere, roadside environments constitute an important landscape element by providing structural and functional connectivity for many plants and animals (e.g., Bennett, 1990; Spooner, 2015). Nevertheless, disturbances often occur in such environments, which can both demote or promote species, depending on the frequency and intensity of given disturbance regimes (e.g., Forman and Alexander, 1998).

In the study, soil disturbances seem to provide ideal nesting and foraging habitat for certain ant species (e.g., *Iridomyrmex* spp.) to thrive in roadside environments. As a result, increased range expansion for myrmecochorous plants could occur through greater dispersal distances provided by such ants along roads. The adaptability of residing ant species to soil disturbances, and their competitive interactions, will influence the success of this mutualism. For example, the seed dispersal and nesting activity of *Melophorus* and *Iridomyrmex* in soil disturbed zones show the adaptability of certain ant species to prevailing habitat conditions in roadside.

Infrequent, long distance dispersal to suitable sites, may be highly important for seedling recruitment in disturbed or modified habitats (Giladi, 2006; He et al., 2009). The extent to which various seed disperser ant species contribute to plant population recruitment and structures requires further investigation of patterns of seedlings establishment in relation to nest sites.

ETHICS STATEMENT

This study was carried out in accordance with AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and Charles Sturt University Animal Care and Ethics Committee policy. This project was exempt from approval requirements because it was deemed "research with limited impact" (category 4).

AUTHOR CONTRIBUTIONS

ZP, PS, and WR conceived and designed the field experiments. ZP performed the experiments. ZP and WR analyzed the data. ZP, PS, and WR wrote the manuscript.

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Large Extent Volunteer Roadkill and Wildlife Observation Systems as Sources of Reliable Data

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Large-extent wildlife-reporting systems have sets of goals and methods to facilitate standardized data collection, statistical analysis, informative visualizations, and use in decision-making within the system area. Many systems employ "crowds" of volunteers to collect these data at large spatial extents (e.g., US state or small country scale), especially along roadways. This raises the important question of how these systems could be standardized and the data made broadly useful in ecological and transportation studies, i.e., beyond the system area or goals. We describe two of the first and longest-running systems for volunteer observation of road-associated wildlife (live and dead) at the US state scale. The California Roadkill Observation System (CROS, http://wildlifecrossing.net/california) uses a form-based data entry system to report carcasses resulting from wildlife-vehicle collisions (WVC). Operating since 2009, it currently (June, 2017) contains 1,338 users and >54,000 observations of 424 species of ground-dwelling vertebrates and birds, making it one of the most successful examples of crowd-sourced, roadkill and wildlife reporting. Its sister system, the Maine Audubon Wildlife Road Watch (http://wildlifecrossing.net/maine) has a similar structure, and can accept data from transect surveys, animal tracks and scat observations, and reports of "no animal observed." Both systems can operate as web-applications on a smart-phone (using a web browser), providing the ability to enter observations in the field. Locational accuracy for California observations was estimated to be ±14 m ($n = 552$ records). Species identification accuracy rate for observations with photographs was 97% ($n = 3,700$ records). We propose that large extent, volunteer systems can be used to monitor wildlife occurrences along or away from roads and that these observations can be used to inform ecological studies and transportation mitigation planning.

Keywords: volunteer science, roadkill, wildlife-vehicle-collision, informatics, crowdsource

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INTRODUCTION

Monitoring biodiversity and investigating causes of changes in biodiversity allows society to make decisions about conservation (Wilson, 1999; Devictor et al., 2010; Bang and Faeth, 2011; Corona et al., 2011) and improve management of human-wildlife conflict. Involving society directly in scientific investigation can transform science from an exclusive process, remote from peoples' day-to-day experience, to one that includes millions of new environmental data collectors (Goodchild, 2007) and is participatory and has immediate relevance and value (Ceccaroni et al., 2016). Projects involving citizen/volunteer scientists have grown considerably in recent years (Silvertown, 2009; Conrad and Hilchey, 2010; Roy et al., 2012), providing data collection at large geographic scales (Devictor et al., 2010), that are often of high-quality (e.g., Ratnieks et al., 2016), have been found to be useful for species-distribution modeling (e.g., Mair et al., 2016), and help connect people to nature and conservation problems (Cooper et al., 2007; Devictor et al., 2010). Sub-national and national governments including transportation organizations (e.g., Harris et al., 2016), increasingly are recognizing the importance of volunteer-collected information (e.g., Bowser and Shanley, 2013 and the Federal Crowdsourcing and Citizen Science Toolkit, https://crowdsourcing-toolkit.sites.usa.gov/).

Volunteer scientists play an important role in understanding the changing distributions of biodiversity (Mair et al., 2016). Their role as sensors can multiply the geographic extent of observations many-fold compared to academic or governmental scientific investigations, while providing data of comparable quality (Ryder et al., 2010; Haklay, 2013). While operating across large geographies (US states), volunteers can maintain observations across broad taxonomies and large spatial extents. In addition, they form both an immediate constituency for science and conservation as well as a face on science that other members of the public may relate to more easily (see Ceccaroni et al., 2016). However, although this position in conservation may be typical (Ceccaroni et al., 2016), the authors are not aware of a demonstration of the effectiveness of volunteer science in changing how natural systems are managed. In particular, transportation organizations have not embraced volunteer-collected data, possibly because of a lack of published evidence of their potential quality.

Informatics is a discipline that provides tools useful to collect, manage, and use diverse types of data to support research and management. The field of web-based environmental informatics has recently evolved in order to assist with large scale environmental analyses, data management, data contributions from disparate sources, and decision-support (Reichman et al., 2011). Volunteer science oriented informatics is an emerging area of practice that could support ecological research, provided broadly-accepted rules are used for developing and sharing controlled vocabularies, data storage models, and metadata. At the same time, emerging social-network, and data-mining methods are greatly increasing our abilities to classify data, estimate trustworthiness, statistically model geographic distribution from point observations, and to provide data directly to rapid response and long-range policy-making. When combined with successful recruitment of volunteer scientists, informatics is critical in providing a transparent, extensive, scalable, and accurate observation system to capture earth processes.

According to Silvertown (2009), there are three factors that have caused the explosion of projects which involve participation of volunteer scientists. The first is the availability of technical tools, including the web, and mobile devices. The second factor is the free labor and the ability to partner with conservation groups. The third factor has to do with public accountability and how these studies provide an opportunity for tax-payers to participate in activities for which their taxes are being used. It is an investment of time toward the advancement of human knowledge and promotes public education of the project's subject matter. As volunteer science projects grow in scope and participation, the need for innovative tools will become greater (Bonney et al., 2009); for example, "eBird" (http://ebird.org) provides multiple tools, including online observation reporting, for volunteer ornithologists (Sullivan et al., 2009). An important aspect of some of these systems is that a social network (connected group of people) may be intentionally or accidentally formed, which may reinforce the collection, and use of the environmental data. Despite the advances these systems have made, there are no broadly-accepted standards and rules for large-extent observation systems focused on terrestrial vertebrates. One purpose of this study was to create and test a rule-based system at large spatial extents.

As with any new technical field, there are challenges associated with building environmental informatics systems to support volunteer-based wildlife observations. A principal informatics challenge is that, although there are many, varied, online systems for volunteer scientists, there is no commonly-used set of rules for data collection, data management, and data visualization. New online systems for recording volunteer observations seem to be built upon a novel set of guidelines, making integration of data from the systems challenging. Although taxonomically-broad systems exist that provide the general public with a place to report nature observations, the rate of reporting is often low for local geographic areas or project and the institutional sponsors may not transform the observations to better societal understanding of the subject matter. This is in contrast to systems built around long-standing social networks of taxonomically-focused naturalists (e.g., http://ebird.org) or for narrow geographic ranges (e.g., http://i90wildlifewatch.org).

Since the projects described here started in 2009 and 2010, there have been several large systems created around the globe to report wildlife on or near roadways (Shilling et al., 2015; see http://globalroadkill.net for examples). In the US, there is only one other state besides CA and ME that accepts volunteer observations of roadkill (Idaho Fish Wildlife Information System, https://idfg.idaho.gov/species/roadkill). This system contains 35,290 reports of 111 species from 658 observers (data accessed February 14, 2017), including the state Departments of Transportation and Fish & Game, for the whole state (216,632 km^2). Around the world, the largest systems are: the Natuurpunt program "Animals under Wheels" (http://waarnemingen.be), which contains 81,974 records (as of February 15, 2017) for Belgium (30,500 km^2) and the Taiwan Roadkill Observation Network (https://roadkill.tw), which contains 37,415 records from 2,777 contributors (as of February 27, 2017) for Taiwan (35,980 km). In many cases, volunteer-collected observations are used to inform mitigation planning on roadways. For example, the Czech Republic's "Animal-Vehicle Collisions" system (http://srazenazver.cz/en/) uses volunteer observations in automated estimations of statistically-significant clusters of carcasses.

We describe an informatics approach that has successfully included observers across two US states in an observational network for live and dead wildlife occurrences on roadways. We focused on wildlife observed on or near roads because for many people they are most likely to observe wildlife while traveling. In addition, the death of animals on roadways is a direct environmental impact that people experience more intimately and thus they may be more motivated to collect observations about animals that have been killed or that may be killed on roads. Finally, roads and traffic provide a near-constant supply of wildlife "samples" which, although spatially-biased at a local scale, provide data about current and changing wildlife occupancy, and movement in landscapes. The system followed a reproducible approach that allowed verification of observation accuracy and easy access to the data collected for analysis using other available tools. The primary goal of this study was to find out whether or not large extent (i.e., US state scale) systems for collecting volunteer observations of road-associated wildlife are viable and can provide reliable data. The purpose of the data collection was to inform both specific improvements to the transportation system to reduce impacts on wildlife and to provide data for further studies of individual species and communities of wildlife over time across a large area. We propose that systems like these could be used to augment the efforts of state and national wildlife agencies, and in the case of transportation agencies, form the basis of mitigation actions.

MATERIALS AND METHODS

Study Area

Two states were chosen for their geographic separation and available social infrastructure to support enrollment of participants. The California Roadkill Observation System (CROS, http://www.wildlifecrossing.net/california) was launched in August 2009 for volunteer recording of carcass observations on California roads and highways. California has a population of more than 37 million people and >499,000 km of unpaved and paved roadways networked across 411,000 km^2 of varied land cover types, including urban, agriculture, forests, grasslands, and desert. Of these roadways, 225,910 km are major urban and rural roads, and 24,398 km are highways. A similar system was developed in early 2010 for Maine, the Maine Audubon Wildlife Road Watch (MAWRW, http://www.wildlifecrossing.net/maine), to allow collection of both live and dead animal observations on and adjacent to Maine's roads and highways. The system also accepts observations of tracks and spoor, as well as "no animal" observations when monitoring a transect (whether no observations were seen on that day). Maine has a population of 1,328,000 people and >60,600 km of roads, including 10,900 km of highways, across its 84,000 km^2 of forests, wetlands, agricultural areas and townships. Currently (June, 2017), both systems are being actively used.

Volunteer and Institutional Observers

The foundation for the observation network is the individual observer, who contributes records to the system. Two models for participant enrollment were used: (1) email communication with a large cadre of potentially-interested observers using list-serves (California) and (2) personal communication with agency partners, members of conservation organizations, and communication with the general public via newsletters (Maine). In both states, we used existing partnerships with wildlife and transportation agencies to advise us on development of the systems. Observers may also have become aware of the sites through regional and national press coverage beginning in September, 2010 and continuing through 2016. New and continuing participation was encouraged by communicating directly, via email, and indirectly, via posting relevant research, news, and project updates on the project websites, syndicated through Rich Site Summary (RSS) feeds of information as it is updated.

Observers voluntarily enrolled in the system through registration, establishing a relationship between the user, and the various roles and privileges they can hold (**Figure 1**). The possible roles within the observation system were anonymous user, authenticated/registered user, content manager, data manager, and transect observer. Patterns of observer participation (e.g., date of joining, organizational affiliation) were tracked using the information provided at enrollment and subsequent activity on the websites. Professional affiliation was assessed for all observers based upon self-reporting. We calculated active participation rates of volunteers based on the time the user enrolled in the system to their last observation entry. This is one of several ways to calculate the length of time the system is used by a user.

Because institutions increasingly use our system to manage their wildlife observation and carcass data (e.g., National Park Service), we created special interaction modes for them. For example, we automated data exports to institutional users that corresponded to geographic areas or animal species. We also fulfilled *ad-hoc* data requests on an approximately monthly basis to transportation, land management, consultant and academic staff, and scientists. These activities generated a feedback loop where more interest was generated as more information was shared.

Informatics

A physical instance of the informatics model was constructed using Drupal (an open source PHP-based web framework), with a MySQL/MariaDB back-end relational database. The web-system was designed to be usable on a wide variety of devices, including smartphones, and tablets (through a browser). The informatics model's primary elements, or concepts, were implemented as "content types." These user-defined objects hold the data associated with the model's concepts, such as observer, observation, and animal taxon, and have various relationships to each other (**Figure 1**). Each object was designed to be generic so that it could be used in other geographic areas, or for other environmental informatics purposes. The informatics model was abstracted to a set of attributes which become the basis for data sharing and conducive for analysis.

The observation record included several key fields that "identify" the record, and provide enough metadata to support export to other standard-based frameworks, including Dublin Core and (a subset of) Darwin Core (Wieczorek et al., 2012). Being able to identify a data record and show its (potential)

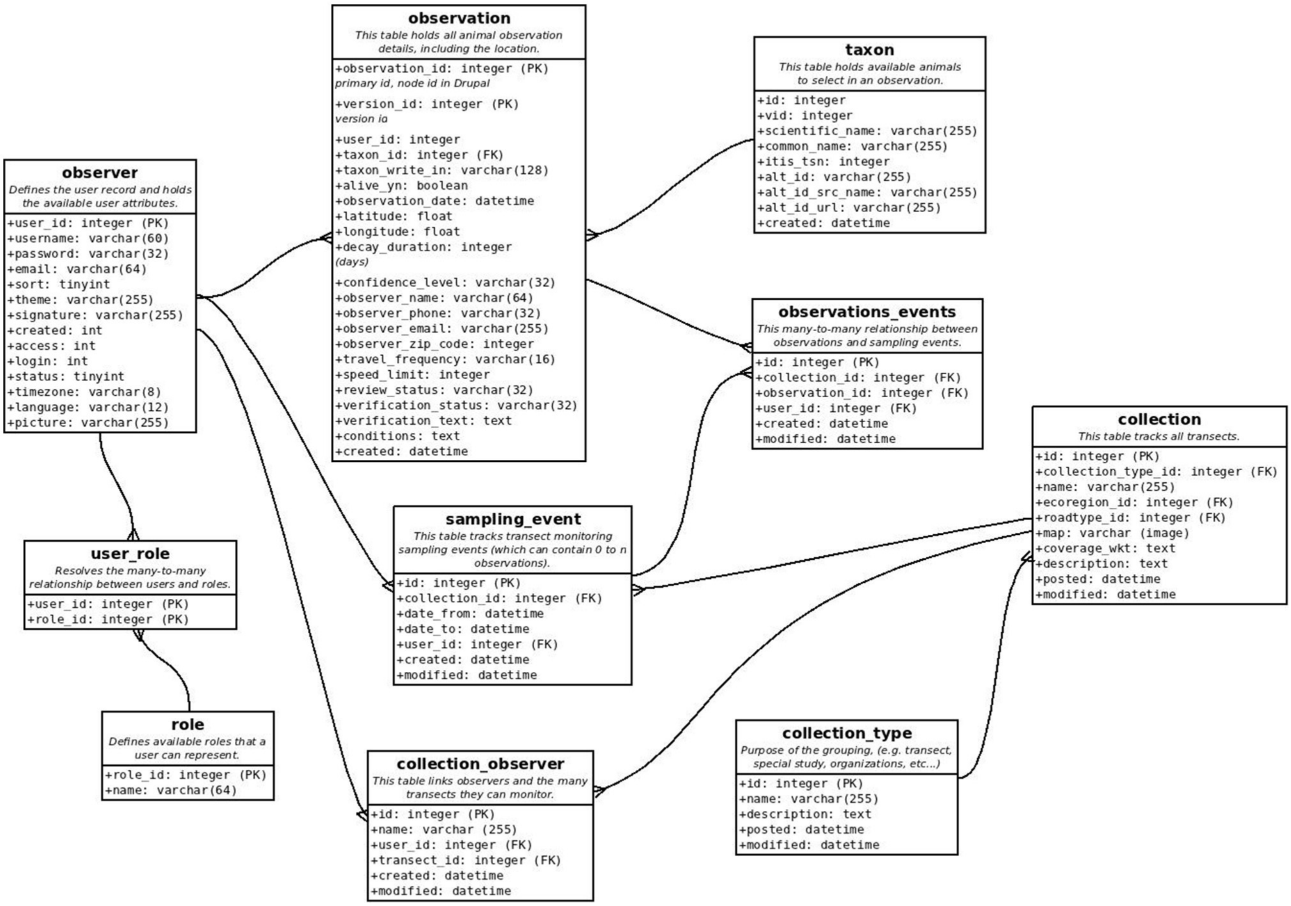

FIGURE 1 | Data model showing relationships among elements in the databases. Lines represent one-to-many relationships. Terms within each box describe the rules and information requirements for each element. Roles and permissions for observers are defined by the site administrators (authors) and are mutable. Controlled vocabularies and standardized ontologies for species are used for all records.

provenance is important detail when using data for scientific analysis, and CROS has been able to preserve any record modifications since the site's inception. This includes capturing the attributes of "who," "what," "where," "when," and "how" for an observation and ensuring these attributes are available when these data are shared (Kelling et al., 2009). These additional fields help provide the context the observation was made, including fields directly related to biology and transportation (**Figure 1**). The preset lists which populate dropdown boxes are called *controlled vocabularies*. Controlled vocabularies used in our model include: species (scientific and common names), species category, road type, habitat type, quality assurance descriptors (e.g., observer confidence), and other customizable attributes. The species records were linked to ITIS (http://www.itis.gov) through a taxonomic serial number (TSN). Depending on the needs of the users, record fields could be required or optional.

All wildlife observations have a spatial context. Geo-location is designated by both latitude-longitude and free-text descriptors for associated roadways and other spatial features, including proximity to structures, or *post miles* (measured markers along roadways). Data entry for location was usually made through an interactive map interface on the website, or by directly entering the latitude and longitude. For some smartphone users, coordinates were indirectly entered using the GPS capabilities of the phones themselves and the coordinates associated with images. The mapping module used the Google API to control the map features such as the zoom bar, the scale bar, the default Google base layers (street, terrain, and satellite view), and the representation of points on the map. The current version of the database does not record estimates of spatial accuracy and precision, though in some cases they can be estimated indirectly.

In the current data model, a single observation permitted only one animal species to be associated with each record. This is useful for later analyses where individual species may be of interest (e.g., mule deer). Surrounding habitat type is an example where multiple vocabulary terms could be used to describe a single observation—an observation could be simultaneously next to an agricultural field, a riparian corridor, and a roadway. The system also provided a facility where observations could be managed based on the species observed and conservation status, stipulated by the IUCN (International Union for Conservation of Nature). For example, observations of two endangered turtle species (ME: Blanding's turtle, *Emydoidea blandingii*, and box turtles, *Terrapene* spp.) and two vulnerable species (ME: spotted turtle, *Clemmys guttata*, and Wood turtle, *Glyptemys insculpta*) were automatically hidden from public view to protect turtle populations from illegal collecting.

Observation Accuracy

Observation confidence was described by a variable where the observer could self-report their confidence in the species identification for their own observation. There were three terms to choose from: 100% Certain, Somewhat Confident, and Best Guess. Since registered observers also entered information about their expertise, such as job title, organization, and experience, data users can compare the observer's expertise with the measure of confidence of an observation, and then make their own assessment of likely data quality. After the observation was entered, a data reviewer could modify the record, and assign reviewer-specific vocabulary terms, which included the options of reviewed, not reviewed, and rejected. Data reviewers included the authors and student interns, who were not responsible for species identification. Species-level accuracy was verified for records with photographs by comparing the photograph with standard pictures of individual species. Species identification was conducted by co-author Shilling and the former natural history curator of the Oakland Museum of California (Dr. Douglas Long), with consultation with other professional biologists as needed. A critical concern for datasets collected by volunteer scientists is about accuracy of the location of the observation or event. We estimated the locational accuracy of a subset of records by measuring the perpendicular distance between coordinates entered by the user during creation of the record (usually by placing a point on a map) and coordinates contained within the Exif data associated with images captured using smartphones with GPS enabled.

RESULTS

Data Collection and Sharing

Observations were collected on all except two of California's 244 numbered highways and across the majority of the state (**Figure 2A**). For Maine, there was a greater concentration of observations in the southern half of the state (**Figure 2B**), reflecting population density. Between August, 2015 and June, 2017, we met 18 requests for observation data for the CA system from individuals, academia, consultants, and Caltrans. These ranged in type from all data for a specific state highway, to all data for a single group (e.g., herpetofauna) or species. For eight partner organizations focused on a single species, we created queries on the web-system, which when clicked resulted in an up-to-date, downloadable data object for the partner.

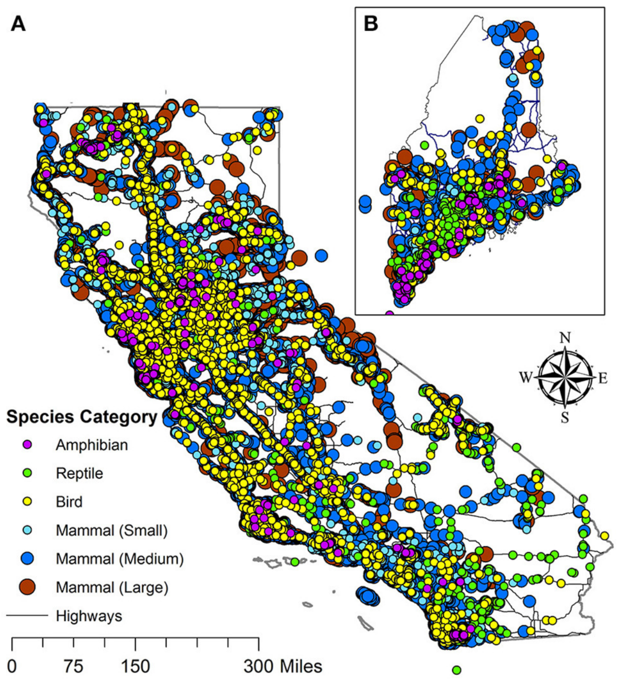

FIGURE 2 | Distribution of all wildlife observations (circles) in California **(A)** and Maine **(B)**.

Volunteer Scientists

Rates of participation and cumulative enrollment varied in response to periodic email contact from system administrators (the authors) and media attention for the project. Rates of new member enrollment in California (CA) were greatest immediately after the authors contacted individuals and organizations throughout the state in September, 2009 and following a New York Times article and National Public Radio stories about the observation systems in early September, 2010 (**Figures 3A,B**). Rates were similarly high after Maine Audubon contacted organizations in Maine (ME) in March, 2010 (data not shown). Following initiation of the system and a large media event, new enrollment rapidly and temporarily increased to ∼150 (CA) and ∼40 (ME) new members/month. The number of observations per year declined through the 7 year period ($P < 0.05$, t-test of slope significance). At the same time, both the number of observations/observer and the number of observations/observer-month significantly increased ($P < 0.05$, t-test of slope significance). Besides observed spikes in new CA members in September of 2 years, there was no effect of month of the year on rate of new member participation (**Figure 3B**), as was also the case in ME (data not shown). When media as a method of contact could not be deployed, rate of new enrollment in CA was 5–10 per month through February, 2017 (**Figure 3A**). Spikes in observations in Spring and Fall (**Figure 3B**) may correlate with increases in emergence of wildlife young and seasonal migrations at these times of year.

Between September, 2009 and June, 2017 (CA) and between March, 2010 and June, 2017 (ME), 1,338 and 622, respectively, observers enrolled in the state systems. The average active participation time (±95th % confidence interval) of observers was calculated as the time between registration and most recent contributed observation and was 446 (±62) out of

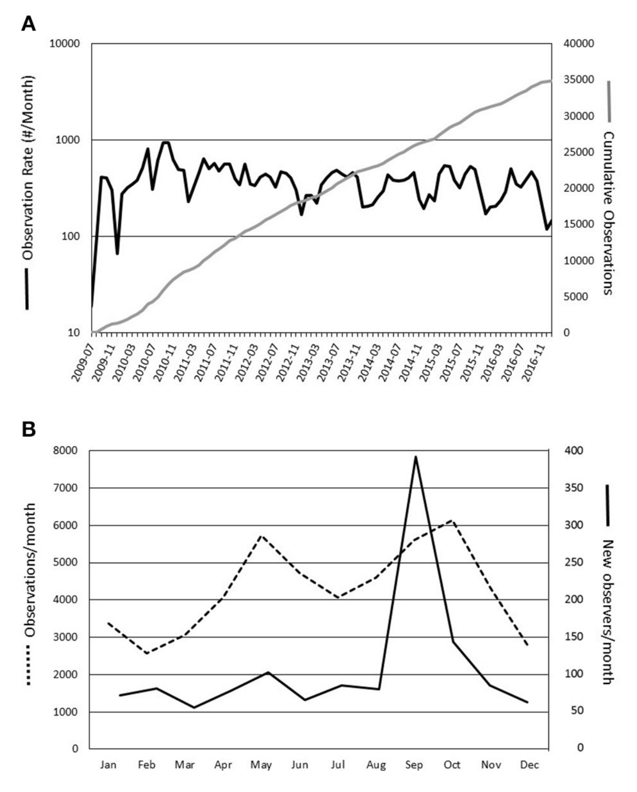

FIGURE 3 | (A) Monthly participation rate (dark line) and cumulative participation (gray line) for the California system between 9/2009 (initiation) and 1/2017 (present). **(B)** Rates of observation per month (dashed line) and new observers per month (black line) for the California system (9/2009–1/2017). Only observations since the start date of each state-system are shown; there were records entered in each system by observers who had made observations of wildlife prior to the beginning of the system.

a possible 1,798 (±74) days (CA) and 353 (±68) out of a possible 1,411 (±72) days (ME). Many of these observers were professional biologists (agency, consultant, or academic or natural historians). Thirty six percent of CA observers self-identified as professional scientists affiliated with agency, academic, and private organizations. The remainder was composed of professionals associated with conservation NGOs (9%), or schools, local government, and un-affiliated individuals (5%). Six percent of ME observers self-identified as affiliated with public or private scientific organizations, 23% were affiliated with natural history organizations (primarily Maine Audubon), and non-university schools, and the remainder provided no affiliation information. In CA, the top 248 contributing observers (excluding the CA Department of Transportation) have contributed >90% of the total observations to date, suggesting that an enthusiastic core group may have developed.

Observation Characteristics

By February, 2017, observers in California had contributed 39,576 observations of roadkilled animals, 2,198 of which were observations that had been recorded before the advent of the system. An additional 14,456 observations came from one organizational contributor—the CA Department of Transportation. Observers in Maine had contributed 4,162 observations of dead animals, 1,968 observations of live animals, 172 observations of "wildlife tracks and signs" and 288 "No animal" observations. The largest group of animals observed by volunteers in both states was medium-sized mammals (CA: 42%, ME: 37%), followed by small mammals (CA: 24%, ME: 30%), birds (CA: 16%, ME: 16%), then small but relatively equal numbers of large mammals (CA: 9%, ME: 5%), reptiles (CA: 6%, ME: 7%), and amphibians (CA: 3%, ME: 3%). In CA and ME, respectively most observations were associated with county paved roads (22, 36%) and state highways (55, 35%), with speed limits of 55 or 65 mph, and on roads where observers drive several times per week (48, 72%) or weekly (18, 12%). Observations were somewhat evenly distributed across seasons, with the highest rate of recording tending to be in the Fall and the lowest in the Winter, with this pattern being more pronounced in ME than in CA (**Figure 3B**). Observations were also unevenly distributed throughout the states, with gaps in certain regions and highways and highest concentrations of observations near urban areas (**Figure 2**).

Observation Accuracy

Observers characterized their confidence in their own animal identification accuracy using three ratings for high (100% Certain), medium (Somewhat Confident) and low (Best Guess) confidence. In CA, 99.5% of records included a self-assessment (by the observer) of confidence in their species identification. Of these, 84% of volunteer observations were assessed as high confidence, 12% as medium confidence, and 4% as low confidence. Similarly in ME, 78% of the observations were characterized as high confidence, 17% as medium confidence, and 6% as low confidence.

Approximately 13% (CA) and 3% (ME) of observation records contained photographs of the dead animal. Because of the low rate of photograph uploads in ME, accuracy was not assessed. Each photograph in CA was examined and compared to online natural history resources appropriate for each animal species. Animals in 11% (CA) of photographs were not identifiable by the authors, but species had been recorded by the observers. We found an additional 6% of records where species of animal had not been identified by the observer to actually be identifiable to the species level. A subset of photographs ($n = 3,700$) with identifiable animals was analyzed for species identification accuracy. We found 97% were correctly identified by the observers. Incorrect species ($n = 111$) identities came from 13 observers, of which ∼50% ($n = 52$) were by one observer, who had contributed >1,800 observations. The incorrectly-identified animals included 33 species, with the most commonly misidentified species being Western Gray Squirrel ($n = 12$) and the most commonly misidentified groups being birds ($n = 30$) and small mammals ($n = 28$).

Locational accuracy was measured for a subset of observations ($n = 552$) with images with embedded location information contained in Exif metadata (e.g., from an iPhone with GPS enabled). Perpendicular distance was measured between the automated location from the smartphone image and the location identified by the same observer placing an observation point upon a map. The median distance from the image-based location and the form-based location was 13.6 m, with 20% of differences being <1 m and 6% >1 km. The rate of large errors (>1 km difference) dropped from 9% of images in 2011 to 0% in 2016. We were not able to determine if the source of the difference between location mapped by the user in the online system and the location obtained from image Exif data was due to observer error, or failure of the user-activated phone to locate itself accurately using GPS or cell-tower triangulation.

DISCUSSION

We demonstrate that formal and accurate reporting of occurrences of wildlife across a broad taxonomic range can occur at the scale of US states, using standardized informatics methods, and validation protocols. The almost perfect species-identification accuracy (97%) may be high enough for most or all management and analytical purposes. This accuracy rate is certainly at the high end of the range for volunteer scientists and trained observers with broad taxonomic target sets (Delaney et al., 2008; Somaweera et al., 2010; Gardiner et al., 2012; Tillett et al., 2012; Ratnieks et al., 2016). Within each state (CA and ME), the systems described here represent the most extensive and taxonomically-broad terrestrial vertebrate monitoring effort, providing information about herpetofauna, birds, and mammals. An important quality of our systems is that even with a rather restricted data domain (road-associated wildlife observations) and very limited budget, we not only were able to attract enough people to create a useful dataset, but also received a high proportion of apparently correct entries from a population with a moderate proportion of professional qualification. Opportunistic wildlife observations in our systems may provide the raw data for presence-only wildlife distribution modeling, statistical analyses of proximate contributors to wildlife-vehicle collisions, minimizing road impacts on wildlife movement, potentially extensive validation of wildlife-distribution models, and estimation of contemporary effects of climate change. Directed censusing of wildlife at index sites using consistent methods and levels of effort (e.g., as conducted by Maine Audubon using our ME system) could provide a mechanism for measuring changes in presence/absence over time (years) and, in the case of wildlife-vehicle collisions, could also provide a way to calculate minimum estimates of the impact of this cause of animal mortality.

Volunteer Scientists

We demonstrate here that a network of volunteer observers can be established at the US state-scale and provide wildlife occurrence information which is useful in understanding impacts of transportation and carrying out certain ecological studies. We found that the availability of a web-system encouraged a large and diverse population of volunteers to report observations and to stay retained as observers. In CA we observed a gradual downward trend in rates of observation, which could be related to a persistent drought in California (i.e., causing declines in wildlife) or the level of engagement with volunteers, such as with face-to-face interactions, which are important for volunteer engagement (Cappa et al., 2016). We also found that the rates of identification-accuracy were high at the species level, which may be related to the high rate of involvement of professional scientists in our systems. It will be worth re-examining these findings periodically to see if the systems are sustainable and contribute valuable scientific information.

Environmental Informatics

We demonstrated that rule-based environmental informatics could be combined with state-scale volunteer observer networks to provide important data about biodiversity and impacts from traffic. Volunteer science-based informatics tools provide repositories for collective knowledge and databases that may be useful in understanding environmental issues across large geographies. For environmental informatics systems, there is a general need for more standardized data access, so that other data-intensive research programs can utilize these data, either through a graphical user interface (for humans), or a machine accessible format for automated processes (Horsburgh et al., 2009). Using observation systems such as the ones we have developed and described at continental scales and broad taxonomic sets will permit exploration of spatial distributions and interactions across scales heretofore unavailable to ecological and biogeographic research (Dickinson et al., 2010). This is recognizing that spatial distributions and activities of volunteers may inject spatial bias in observation richness, which would need to be controlled for Geldmann et al. (2016). A critical need is for academic and agency data-users to encourage and support this type of approach, which will radically expand the data available for wildlife management and conservation.

Observation Accuracy

One possible concern about volunteer science observations is that they will tend to be biased and inaccurate. In limited studies, volunteer scientists have been shown to provide observations that are of verifiable and comparable quality to institutional scientists (Fore et al., 2001; Davis and Howard, 2005; Ryder et al., 2010; Kremen et al., 2011; Ratnieks et al., 2016). For example, for bird nest survival rates in Washington D.C., models that incorporated observer effect showed no significant difference in monitoring nests between Smithsonian biologists and volunteer scientists (Ryder et al., 2010). In the present study, observers self-reported high levels of certainty (84% high confidence) in their own observations and for the most part were correct in that assessment, with accuracy of identification to the species level of 97% for records with associated photographs. That being said, the potential exists that observations with photographs are somehow different from those without and accuracy rates could be different between observations with and without photographs. The rate of accuracy reported here is comparable to the highest reported in the literature, which are usually for trained volunteers (e.g., Ratnieks et al., 2016).

Locational accuracy is very important for wildlife observations, especially of roadkilled animals (Gunson et al., 2009) where accuracy rates of <1 km are important in developing models of causes of roadkill (Kinley and Newhouse, 2009). We did not field-verify location accuracy of live or dead wildlife observations; however, we did find that observers who took pictures of carcasses accurately placed observations on a dynamic map, relative to the position obtained from smartphone GPS. Although this indicator of spatial accuracy of roadkill observations is only a surrogate for field-verification, the high median locational accuracy (±14m) suggests care in recording spatial location of the observation. A caveat to the comparison of the two methods for determining observation location is that the GPS on smartphones have a limited antenna, and in complicated settings (e.g., urban, forest, valleys) may require several minutes to communicate with enough satellites to accurately estimate position (Pesyna et al., 2014) which still may be off by 10–20 m (Zandbergen, 2009). The best approach for wildlife observation recording may be a combination of GPS-enabled smartphones and confirmation using online maps.

Participatory Ecological Modeling

Data from the statewide wildlife observation systems described here belong to the community of users who pool their resources into creating a rich observation database and can download the data for their own research. It will be important to plan for maintenance of the large-scale volunteer networks and informatics systems that provide these wildlife observations. It will also be important to encourage integration and compatibility among taxonomically and/or geographically overlapping systems in order to encourage the formation of a "wildlife observation network" that provides scientists, managers, and the public with information about wildlife. These federated, standardized systems of wildlife observations will be instrumental in understanding and monitoring large-scale ecological characteristics and processes, such as extensive, taxonomically-focused monitoring (e.g., Gardiner et al., 2012), conservation success (Homayoun and Blair, 2016; Miller et al., 2017), changes in species distributions (Mair et al., 2016), mammalian invasions (Maistrello et al., 2016; Courchamp et al., 2017), and changes in species occurrence and abundance in response to climate change.

Governing Systems Decision-Support

Wildlife monitoring across broad taxonomies is seldom carried out at the US state scale. Doing this requires a standard data collection and management protocol and a group of people funded or willing to do it. We propose a volunteer science and informatics-based approach, as described here, to augment often-underfunded state and federal agency efforts. The system should support expanded and continued volunteer enrollment, to allow for turn-over, and include verification approaches to ensure data quality. The resulting data could be of high enough quality to become an important part of each state's corporate database for wildlife distributions. In the case of live and dead wildlife observations along roadways, this would aid in developing mitigation strategies for potential and actual wildlife-vehicle collision hotspots (Shilling and Waetjen, 2016). An added advantage of instituting crowd-sourced data collection associated with transportation is to introduce democratic process to what is a traditionally closed decision-loop. This democratization is likely to increase sustainability and public inclusion (Irwin, 1995; Bäckstrand, 2003), a stated goal of most transportation organizations and other government agencies.

While this study addresses assessing and mapping wildlife observations along roads, the informatics approach is broadly applicable to detecting anthropogenic changes in other natural systems. In many cases, such as new infestations of invasive species or communicable diseases, rapid response is needed, but early detection is unlikely without a network of observers much wider than the professional communities (Peterson and Vieglais, 2001 tell this story for the Asian Longhorn Beetle). The common features include geographically-widespread systems and attributes, who-where-what-when-how components of observations, sudden events, and amateur enthusiasts.

Guidelines for Similar Systems

The following are guidelines for the development and implementation of wildlife-observation systems relying on volunteer contributions. They are based on our experience over the last 8 years with the systems and volunteers described here.

(1) Start with an idea of possible uses of the data being collected. In our case, we wanted to inform both specific improvements to the transportation system to reduce impacts on wildlife and to provide data for long-term studies of individual species and communities of wildlife across a large area.

(2) Use standard data formats, types, and informatics model and keep data entry forms as simple as possible. We provide a data model here based on the common need for "where," "what," "when," "why," and "who" types of information about observations. We suggest anyone designing a new system use existing systems such as ours to organize data and develop aspects such as controlled vocabularies for completing observation forms.

(3) Begin with and continue with a plan to communicate with and include volunteers. Recruitment and retention of volunteers is the cornerstone for successful observation projects and is most easily done by showing the use of data in management (see below), and mention of the system in news media.

(4) Periodically demonstrate utility of the observation data to meet project goals. Volunteers are often involved in order to contribute to wildlife conservation and will stay interested if their data are being used and they understand the need for their contributions. In addition, for ecologists, engineers, and others to trust and use the data, the accuracy and reliability of the data should be demonstrated.

In addition to these steps, to construct a service similar to those described here, one needs: identification materials for the target system (here, wildlife species), an interactive mapping service, a set of menu choices for the attributes that will be most useful in interpreting and filtering observations, capabilities for uploading, and managing photographs, and methods for providing satisfaction, data-sharing, and visualizations for volunteer observers. With these features such a system is scalable, relatively interoperable with global datasets such as the Global Biodiversity Information Facility and readily combined with other mapped systems to estimate risks and benefits of alternate management and policy approaches over large landscapes.

AUTHOR CONTRIBUTIONS

Both authors contributed to the conception and design of the work described, shared in drafting and revising the manuscript, approved of the final version, and are equally accountable for all aspects of the work.

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Testing the Value of Citizen Science for Roadkill Studies: A Case Study from South Africa

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Roads impact wildlife through a range of mechanisms from habitat loss and decreased landscape connectivity to direct mortality through wildlife-vehicle collisions (roadkill). These collisions have been rated amongst the highest modern risks to wildlife. With the development of "citizen science" projects, in which members of the public participate in data collection, it is now possible to monitor the impacts of roads over scales far beyond the limit of traditional studies. However, the reliability of data provided by citizen scientists for roadkill studies remains largely untested. This study used a dataset of 2,666 roadkill reports on national and regional roads in South Africa (total length ∼170,000 km) over 3 years. We first compared roadkill data collected from trained road patrols operating on a major highway with data submitted by citizen scientists on the same road section (431 km). We found that despite minor differences, the broad spatial and taxonomic patterns were similar between trained reporters and untrained citizen scientists. We then compared data provided by two groups of citizen scientists across South Africa: (1) those working in the zoology/conservation sector (that we have termed "regular observers," whose reports were considered to be more accurate due to their knowledge and experience), and (2) occasional observers, whose reports required verification by an expert. Again, there were few differences between the type of roadkill report provided by regular and occasional reporters; both types identified the same area (or cluster) where roadkill was reported most frequently. However, occasional observers tended to report charismatic and easily identifiable species more often than road patrols or regular observers. We conclude that citizen scientists can provide reliable data for roadkill studies when it comes to identifying general patterns and high-risk areas. Thus, citizen science has the potential to be a valuable tool for identifying potential roadkill hotspots and at-risk species across large spatial and temporal scales that are otherwise impractical and expensive when using standard data collection methodologies. This tool allows researchers to extract data and focus their efforts on potential areas and species of concern, with the ultimate goal of implementing effective roadkill-reduction measures.

Keywords: spatial clusters, mammals, mortality, road ecology, wildlife-vehicle collisions

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INTRODUCTION

Roads (and their associated users) affect wildlife through a wide range of mechanisms. They are responsible for habitat loss, degradation (Trombulak and Frissell, 2000), and decreased landscape connectivity resulting in a barrier effect and road avoidance behavior (D'Amico et al., 2015a). Furthermore, these fragmented habitats act as filters, allowing some species to cross while others are killed (e.g., snake species that cross roads at low speed are more at risk of mortality than others that cross rapidly, Andrews and Gibbons, 2005). The most conspicuous and studied effect of roads on wildlife is the direct casualties resulting from collisions with vehicles (i.e., roadkill). Wildlife mortality due to roadkill often exceeds natural rates (Forman et al., 2003) and has the potential to affect all individuals in a population equally, unlike predation (Jaarsma et al., 2006). For some species, such as the Florida panther (*Felis concolor coryi*), vehicle collisions are the main cause of mortality (Harris and Scheck, 1991). In certain cases, it can even be the cause of population decline, for example, causing a decrease of 30% in hedgehog (*Erinaceus europaeus*) density in The Netherlands (Huijser and Bergers, 2000).

In recent decades, the number of studies focusing on the impacts of roads on biodiversity has increased considerably, leading to the rise of an applied scientific discipline called "road ecology" (Forman et al., 2003; Coffin, 2007). Early road ecology research used roadkill to monitor changes in population (e.g., Baker et al., 2004), focused on emblematic species (e.g., Hobday and Minstrell, 2008) or examined spatial and temporal patterns in the distribution of roadkill (e.g., Taylor and Goldingay, 2004; Ramp et al., 2005). Many of these studies relied on road surveys conducted at regular intervals by trained observers (Barthelmess and Brooks, 2010; D'Amico et al., 2015b). Despite providing high quality data, these methods are costly in terms of both time and logistics and can thus only be applied to relatively small areas (Caro et al., 2000; Barthelmess and Brooks, 2010).

Citizen science—a new form of data acquisition involving public participation - potentially provides a large pool of enthusiastic contributors that could enhance data collection at scales far beyond the limit of traditional field (Wilson et al., 2013). Globally, dozens of web-based systems for reporting roadkill exist (Shilling et al., 2015). Examples include Project Splatter in the UK (https://projectsplatter.co.uk), the National Wildlife Accident Council in Sweden (http://www.viltolycka.se/) or the California Roadkill Observation System in the USA (CROS - http://www.wildlifecrossing.net/california). These systems vary greatly in purpose and taxonomic focus. While some citizen science projects have a standardized methodology for data collection, most systems allow for the submission of opportunistic or *ad hoc* observations, even though these are perceived to be of lower quality (Bird et al., 2014). Such non-standardized methods of data collection can bias the information and conclusions, as scientists cannot control for research effort, accurate species identification, or an observational bias toward more charismatic species. In the design of roadkill mitigation, it is therefore vital to understand whether informally collected data are sufficiently biased to potentially direct conservationists' attention to the incorrect areas or even species.

Several studies have tried to identify and quantify bias in roadkill data collection (e.g., Slater, 2002; Santos et al., 2011) or develop standardized recording methods (e.g., Collinson et al., 2014), but to our knowledge none has tested the capacity of data from citizen science surveys to provide reliable roadkill data. In this study, we assessed the potential value of citizen science data for roadkill studies by comparing *ad hoc* data provided by citizen scientists (termed "occasional") to that of (1) road patrols by trained personnel (termed "road patrol") and (2) regular, informed observers working in the conservation field (termed "regular"). These data were collated in the first national database on mammalian mortality on South African roads, by the Endangered Wildlife Trust (EWT), from records collected from 2010 to 2015. We compared both the species reported as well as the spatial clustering of roadkill reports between the three reporter types (occasional, regular and road patrol), to assess differences and potential sources of bias. If bias exists in the citizen science data, we expected to find higher frequencies of larger, more common and charismatic species in the citizen science roadkill data (Caro et al., 2000).

METHODS

Data Collection

Following the launch of a national roadkill awareness campaign in 2013, the EWT gathered roadkill data using four different approaches: (i) developing a form-based reporting system on a website (ii) soliciting historical records of roadkill incidents (iii) developing a smartphone-application called "*EWT Road Watch*" and (iv) using social media such as LinkedIn, Twitter and Facebook to attract interest. All of these methods relied on data collected by lay people, satisfying the criteria for citizen science. From these four methods, two main types of data collection strategies emerged: occasional/random observations (187 individuals), and data from regular observers (nine individuals) that each provided >50 roadkill reports. Regular observers were not trained in data collection but were working in the Zoology/Conservation sector and thus their data were considered to be accurate. Road patrol staff received annual training (conducted by the EWT) in species identification, collection of roadkill data, and the taking of photographs. Data could only be verified if a photograph was submitted with the report.

In parallel to citizen scientist data collection, road patrol agencies were trained by EWT staff to conduct regular road transects on set routes. Transects were conducted on the N3 highway from Johannesburg to Durban along a total length of 431 km, driven four times a day every day (twice in each direction); teams were allocated to six shorter sections to ensure the whole distance was covered effectively. Once discovered, carcasses were removed from the road to avoid recounts (Collinson et al., 2014; Guinard et al., 2015). Road patrols took place from July 2011 to November 2014.

Due to the small number of reports pre-2011 and in 2015, we conducted the following analyses on the data from 2011 to 2014 only.

Identification of Repeated Sampling

Within each group of reporters (regular vs. occasional and road patrol vs. citizen scientists), we defined "repeated sampling" as a roadkill that was reported for the same species within a 2-day period, <150 m from one another. This resulted in two reports from the occasional reporter's dataset and 22 from the regular reporters' datasets being removed from the final dataset. There were no repeated samples by citizen scientists on the N3 since the road patrol removed carcasses from the road once detected.

The final dataset comprised a total of 2,642 roadkill reports, 1,647 from regular reporters, 786 from occasional reporters and 209 from the N3 road patrols (see **Figure 1** for spatial distribution and Supplementary Materials S1, S2). A further 183 roadkill reports from citizens were located on the N3 surveyed by the road patrol.

Data Categorization

Each species in the dataset was categorized according to taxonomic order. Domestic species (cat *Felis catus*, dog *Canis lupus familiaris* and livestock) were pooled into a group labeled "domestic," and unknown/ unidentifiable mammal species were grouped into "Mammalia". Three body mass classes were defined to account for carcass detection probability, using the average adult female body mass for African mammals (Skinner and Chimimba, 2005): very small (<2 kg), small (2–10 kg) and medium to large (>10 kg). Unknown species were assigned to a size class labeled "unknown." Based on the National Red List of mammals in South Africa (Child et al., 2016) each species in the dataset was assigned to a Red List threat category. Domestic species and generic Mammalia were assigned a Not Determined (ND) Red List status.

Data Analysis

Citizen Science vs. Road Patrol Data

To assess the potential of the citizen scientist's contribution to roadkill surveys, we first compared the data provided by citizen scientists (both regular and occasional observers) to that compiled by systematic road patrols. For this analysis, we considered only the citizen science data collected on the road section where road transects were conducted by the road patrols (i.e., the N3 highway). The dataset was thus composed of 183 reports from citizen scientists compared to 209 reports provided by the N3 road patrol (**Figure 1**). We compared species size, taxonomic category and Red List category between these two datasets using Chi-square tests with false-discovery-rate correction for multiple comparisons. Analyses were performed in R software version 3.2.2 (R Core Team, 2016) using the function "chisqPostHoc" from the "NCStats" package (Ogle, 2015).

Regular vs. Occasional Observers' Data

To further assess the value of random roadkill reports, we compared data submitted by regular and occasional observers across South Africa. We used Chi-square tests with false-discovery-rate correction for multiple comparisons to test for difference in terms of size, taxonomic category and Red List category.

Cluster Analysis

To compare the spatial patterns between the three different types of observer (occasional, regular and road patrol), we conducted a cluster analysis using the KDE+ method defined in Bíl et al. (2015). For this analysis, datasets included only roadkill reports that provided a GPS location ($N = 1,836$, 137 for N3 road patrol, 505 for occasional and 1194 for regular reporters). Of these, only a portion could be clearly associated to a specific road from the Open Street Map data (http://download.geofabrik.de/africa/south-africa.html). Our dataset for comparison between trained and untrained observers reporting roadkill on the N3 highway consisted of 137 reports submitted by road patrols and 1874 from citizen scientists respectively. The remaining reports from our dataset provided a comparison of 872 (regular reporters) and 345 (occasional reporters) reports representing the South African road network (total length ∼170,000 km, accounting for ∼25% of the South African road network; Karani, 2008).

RESULTS

From 2011 to 2014, a total of 2,642 mammalian roadkill incidents were reported on roads (totaling ∼170,000 km) in South Africa, comprising 102 mammalian species from 14 orders (Supplementary Material S1). Of the 287 species on the National Red List, 24.7% ($n = 71$) were reported killed. Of these, 78.9% were of Least Concern (LC, $n = 56$), 11.3% were Near Threatened (NT, $n = 8$), 5.6% were Vulnerable (VU, $n = 4$), and 4.2% were Endangered (EN, $n = 3$). A total of 196 observers contributed toward the survey and we identified nine regular observers who provided 67.6% ($n = 1,647$) of the total citizen scientist dataset ($n = 2,433$). The majority of citizen reports were submitted via email (77.2%, $n = 1,879$) and on the smartphone-application (19.5%, $n = 45$). Social media (1.4%, $n = 33$) and direct reporting through SMS or phone call (1.5%, $n = 37$) provided only a small fraction of the data.

Roadkill Patterns Identified from the Citizen Scientist (Occasional and Regular) vs. Road Patrol Data

Along the same section of the N3 highway, trained road patrols and citizen scientists (from both occasional and regular reports totaling 183 reports) reported a total of 31 and 35 mammalian species, respectively, across eight and nine taxonomic groups (Supplementary Material S2). The vast majority of the road patrol reports were of small and medium sized species (**Figure 2A**), in NT and ND Red List categories (**Figure 2B**). The distribution of Red List category ($\chi^2 = 21.7$, $df = 2$, $p < 0.001$) from road patrol reports was significantly different from that reported by citizen scientists. Citizen scientists reported more roadkill of medium-size and species falling in the Red List category EN (**Figures 2A,B**). Additionally, they reported less very-small and NT species (**Figure 2B**). The frequencies of taxonomic groups reported differed significantly between road patrols and citizen scientists ($\chi^2 = 26.9$, $df = 8$, $p < 0.001$), the latter reporting

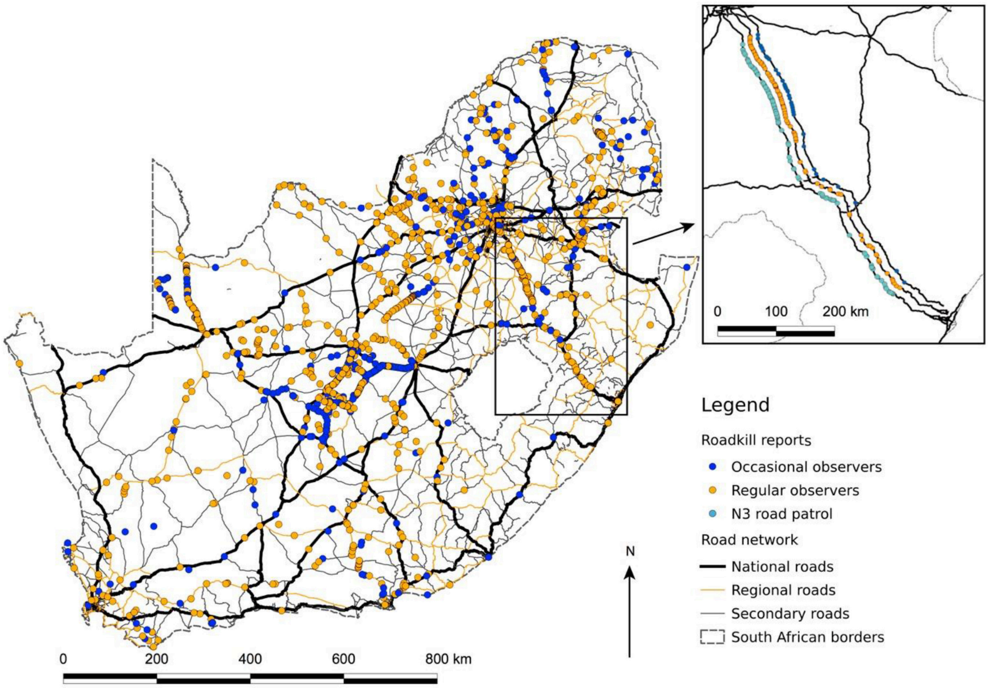

FIGURE 1 | Spatial distribution of roadkill reports collected from occasional (dark blue dots) and regular (yellow dots) reporters in South Africa and along the N3 (insert) where roadkill reports were also provided by trained personnel conducting regular road patrols (light blue dots).

more Carnivora and domestic mammals and fewer Rodentia and unknown mammals than road patrols (**Figure 2C**).

The top three roadkill species reported by road patrols (unknown rabbit sp *Leporidae* sp., 22.1%; domestic dog *Canis lupus familiaris*, 11.5%; and black-backed jackal *Canis mesomelas* 10.5%; Supplementary Material S2) were among the top four species reported by citizen scientists, followed by serval (*Leptailurus serval*, 14.8%).

Using the KDE+ method, we identified eight and 11 significant clusters of roadkill from the road patrol and citizen scientist reports respectively. Using a threshold strength of 0.4 to define biologically relevant clusters, seven road patrol clusters were relevant as were eight citizen scientist clusters. These clusters represent 10.9% ($n = 15$) and 12.1% ($n = 21$) of the roadkill reported by road patrol and citizens respectively. Most of these clusters (four from road patrols and seven from citizens) were located within a section of ∼41 km along the northern part of the surveyed road (**Figure 3**), representing ∼10% of the total distance surveyed (431 km).

Comparison of Regular and Occasional Observers' Data

Taxonomic and Trait Patterns

The body size of the majority of the species reported killed by both regular and occasional observers was small (**Figure 4A**) and of LC Red List category (**Figure 4B**). Carnivora was the order most often reported for both citizen scientist observer types, with Lagomorpha and Rodentia forming the remaining bulk (Supplementary Material S1, **Figure 4C**). There was no significant difference in the frequency distribution of size ($\chi^2 = 0.9$, $df = 3$, $p > 0.05$), Red List categories ($\chi^2 = 2.7$, $df = 3$, $p > 0.05$) or taxonomic groups ($\chi^2 = 18.7$, $df = 13$, $p > 0.05$) reported between occasional and regular observers.

A total of 70 species were reported by occasional observers compared to a total of 88 for regular reporters. In both datasets, scrub hare (*Lepus saxatilis*; regular $n = 169$ and occasional $n = 85$) was the most prevalent species, followed by unknown species (regular $n = 148$ and occasional $n = 72$). Bat-eared fox (*Otocyon megalotis)* ranked fourth ($n = 123$) and third ($n = 66$) respectively for regular and occasional observers, followed by black-backed jackal, which ranked fifth for both regular ($n = 107$) and occasional ($n = 48$) observers. Aardwolf (*Proteles cristata)* ranked sixth ($n = 82$) and fourth ($n = 52$) respectively for regular and occasional observers, whilst unknown rabbit species (Leporidae) ranked third ($n = 127$) in the regular observers' dataset but was sixth ($n = 42$) in the occasional observers' dataset (Supplementary Material S1).

Spatial Patterns

From the regular and occasional reports, the KDE+ method allowed for the detection of 45 and 14 roadkill clusters respectively. Of these, 30 clusters for regular reporters and 9 clusters for occasional reporters had a strength superior to 0.4 (**Figure 5**). These clusters represent roadkill reports from 43.1% ($n = 73$) of regular and 3.6% ($n = 18$) of occasional citizen scientist participants. Nearly half of the clusters from both regular

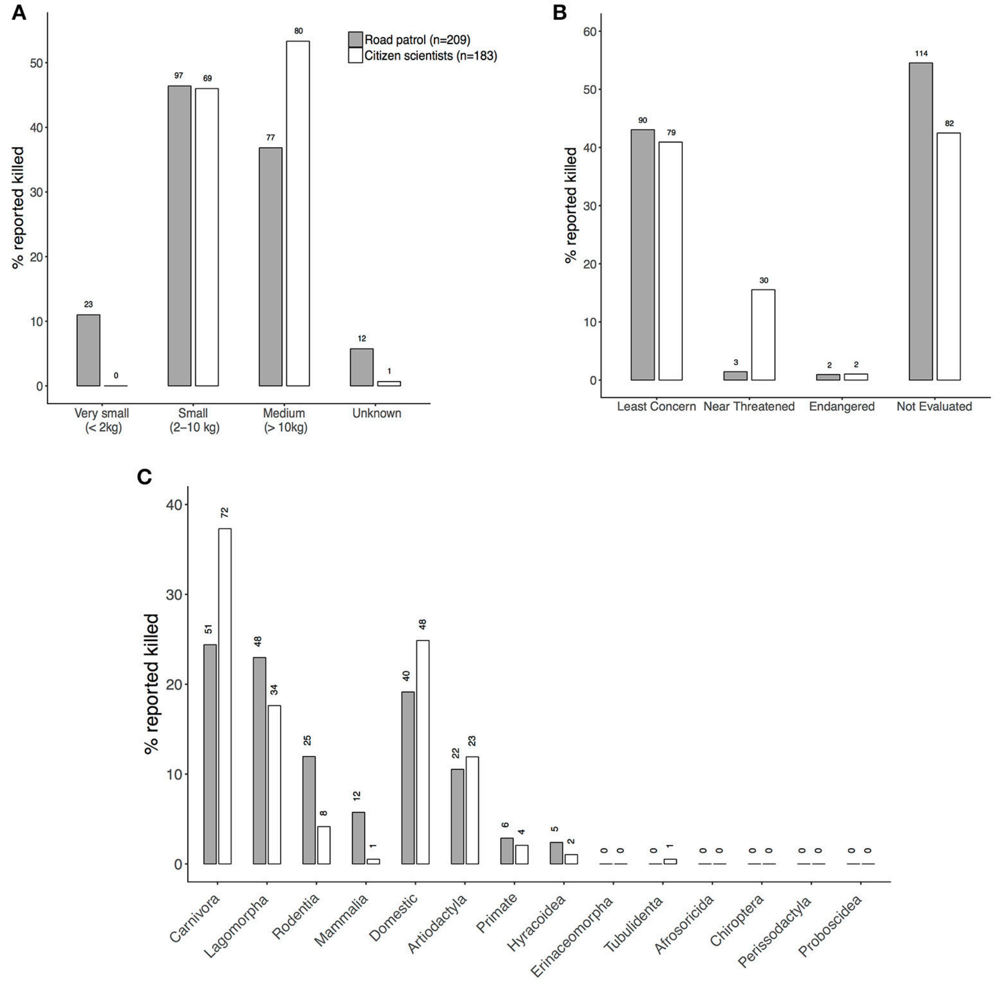

FIGURE 2 | Comparison of the distributions of **(A)** sizes, **(B)** Red List category of species and **(C)** taxonomic groups reported killed along a 431 km section of the N3 road by road patrols (white) and citizen scientists (gray). Figures above bars represent the number reported in each category.

($n = 14$) and occasional ($n = 2$) observers were located in the center of the country (Bloemfontein-Kimberley region; **Figure 5**) along sections totaling ~550 km of both national and regional roads. Four regular observers' clusters were also located on the N3 (**Figure 5**).

DISCUSSION

Summary of Key Findings

Data quality gathered by citizen scientists can be compromised (Floridi, 2012) due to inaccuracies in reporting and lack of scientific understanding (Batini and Scannapieca, 2006) and thus should be interpreted with caution. Our results suggest that data collected by *ad hoc* citizen scientists (i.e., occasional reporters) can be as accurate as those obtained by trained or informed reporters in terms of broad spatial patterns and species identification. Citizen scientists (i.e., occasional and regular reporters) who gathered roadkill data in South Africa between 2011 and 2014, largely agreed with the data from that of trained road patrols. Furthermore, the regular, informed reporters conveyed similar roadkill patterns as the occasional reporters.

Caro et al. (2000) noted during roadkill counts in California that small mammals (<10 kg) are more difficult to see when driving at normal speed (~100 km/h^{-1}). Furthermore, these smaller species are often quickly removed from roads by scavengers or become problematic to identify by observers as they become "flattened" by vehicles using the roads (Hels and Buchwald, 2001), thus making the recording of roadkill less accurate. In the case of our study, South African citizen scientists were less likely to report smaller species than the road patrols along the N3 highway. This is in line with our assumption that citizen scientist data would be biased toward larger species (>10 kg). In addition, citizen scientists might also lose motivation or put less effort in to reporting unidentified or "low profile" species, assuming that the information is not scientifically valuable (Lukyanenko et al., 2016). Our data show that road patrols frequently report a high number of very-small

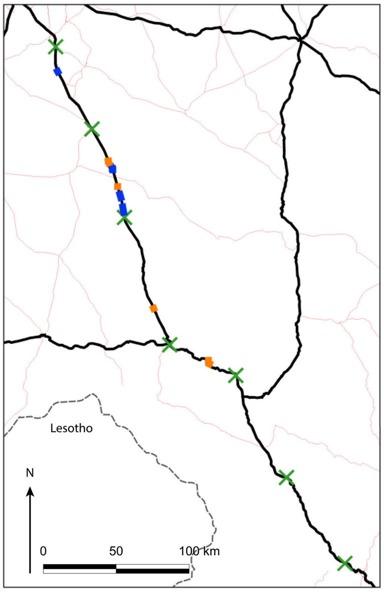

FIGURE 3 | Clusters of roadkill reported on the N3 identified from the road patrol (orange) and citizens (blue) reports using KDE+ method. Only clusters with a strength superior to 0.4 are shown.

and unidentified species. This is likely due to them driving at slower speeds (<60 km/h^{-1}) as well as the training they receive from the EWT in species identification and collecting roadkill data (Collinson, 2016, pers. obs.).

A third of the roadkill clusters identified by occasional reporters were found in locations that agreed with those of regular reporters. The same was true when comparing data from road patrols to both citizen scientist reports (occasional and regular) as well as those of regular and occasional reports. Clusters of roadkill (i.e., roadkill hotspots) were concentrated on the same road sections in South Africa which represents 0.3% of the road network analyzed (~170,000 km). By comparison, 70% of clusters were not close to the clusters identified using data from the other reporting group (road patrol vs. citizens or regular vs. occasional reporters). These most likely resulted from the difference in sampling efforts by both types of citizen reporters. In our opinion, the spatial extent of the area surveyed by the 187 occasional observers (~765,000 km of the entire road network in South Africa; Karani, 2008) is expected to be larger than the one sampled by the nine regular observers (~170,000 km of road); smaller and less utilized roads are likely to have received a different sampling effort from both user groups. Our sample size from the occasional reporters ($n = 505$) was insufficient in terms of monitoring the country's road network, and consequently, a larger sample size is required to enable cluster analysis of the occasional data. We propose that the clusters identified as "roadkill hotspots" could be the focus of further and more detailed study that concentrate on the fine-scale patterns of roadkill and factors potentially responsible for the high intensity of collisions. Thus, while citizen scientists may not identify all the clusters noted by trained observers, these data can provide

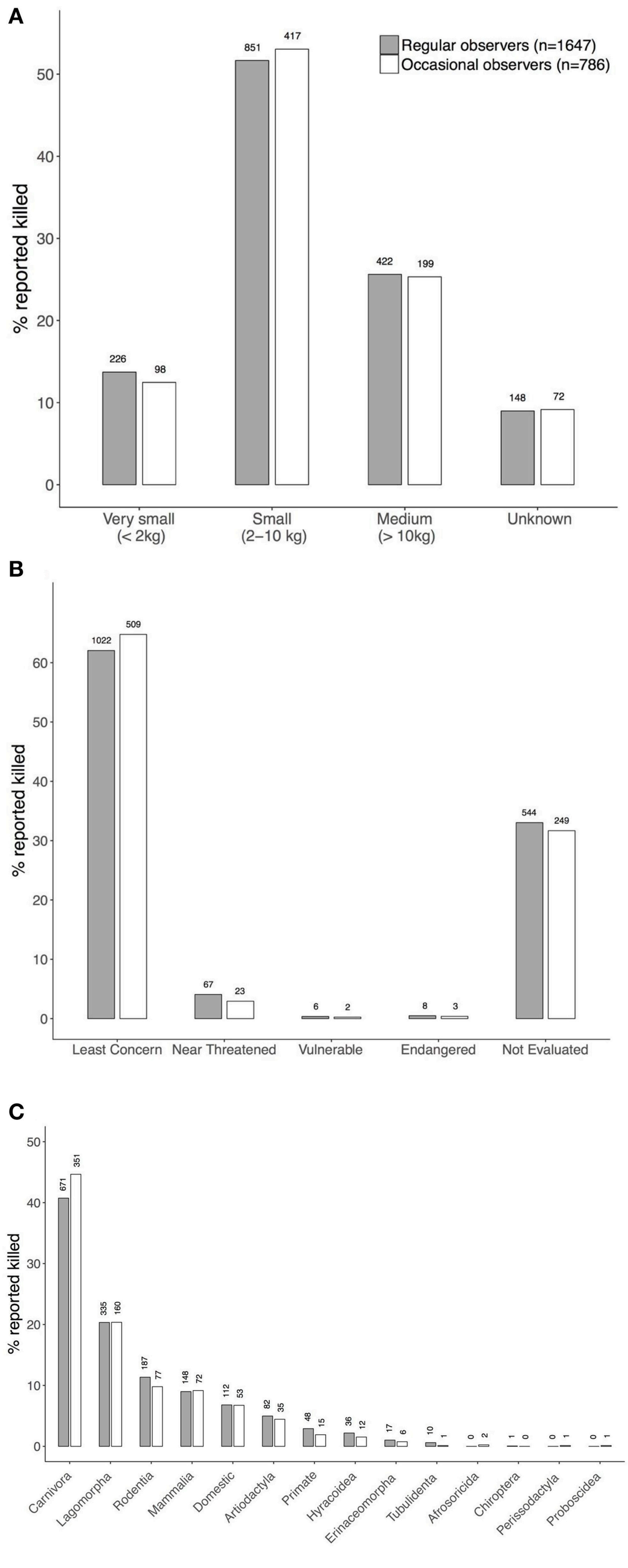

FIGURE 4 | Comparison of the distributions of **(A)** sizes, **(B)** Red List category of species and **(C)** taxonomic groups reported killed across South Africa by regular (gray) and occasional (white) observers. Figures above bars represent the number reported in each category.

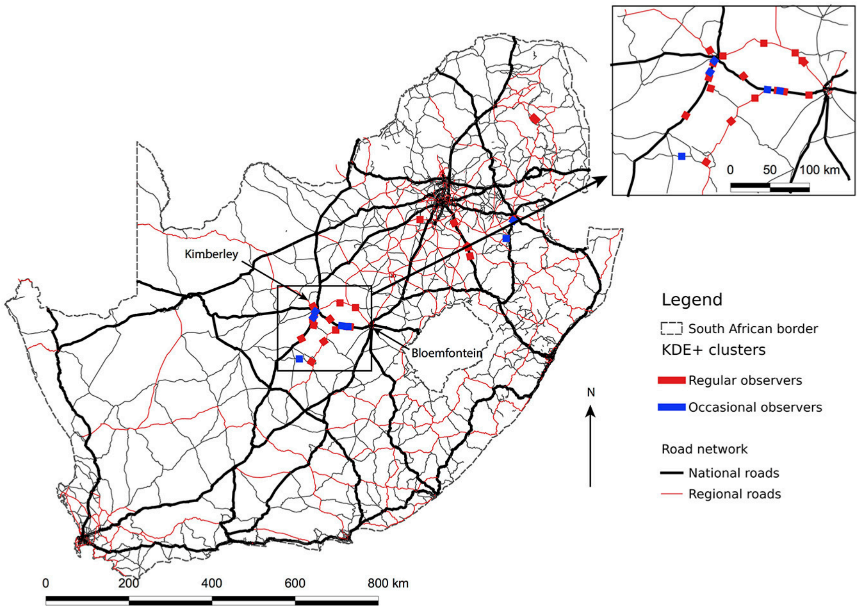

FIGURE 5 | Clusters of roadkill reported across South Africa from regular (red) and occasional (blue) reports using KDE+ method. The insert shows the Bloemfontein-Kimberley region where most of the clusters are concentrated. Only clusters with a strength superior to 0.4 are shown.

an early warning system for potential roadkill hotspots and data management (Shilling et al., 2015).

The role of citizen science in research and monitoring is increasing globally as a data collection tool (Lukyanenko et al., 2016) despite skepticism of the data produced by non-experts (Swanson et al., 2016). Our analysis demonstrates that the assumption that we target only "educated citizens" for the collection of roadkill data (Batini and Scannapieca, 2006) appears to be unnecessary. In this large-scale study of citizen science data collection patterns in South Africa, we conclude that the biases that may be present in our data (Floridi, 2012) are not significant enough to ignore the immense value of citizen science projects. Our study demonstrates that *ad hoc* citizen science has the potential to map roadkill occurrence and identify hotspots in a reliable and robust manner compared to that of trained road patrols and informed reporters. However, we propose that *ad hoc* citizen science data should only be used to identify general patterns or trends. As Lukyanenko et al. (2016) states, "*Citizen science is about writing a story where citizens contribute to the plot. Experienced researchers should then assume the role of directing the actors and writing the dialogue.*"

Recommendations

We outline below recommendations to improve the accuracy, sampling effort and the motivation of the citizen scientist.

(1) Data accuracy: Our dataset did not allow for species identification verification, since not all data were submitted with a photograph. We therefore propose that all similar projects encourage the submission of a photograph that can be verified by an expert, or cross-referenced by other citizen scientists (Swanson et al., 2015, 2016).

(2) Sampling effort: The sampling effort of data collected by both the citizen scientists and the road patrols was not recorded and we were therefore unable to correct for sampling effort bias; this leads to potential bias in both spatial distribution and species reporting. We therefore propose that (where possible) sample effort is encouraged with data submitted on not only where roadkill occurs but also roadkill absence (Shilling et al., 2015). For example, a smartphone application can either automatically record distances driven, or prompt users to report these data.

(3) Training and feedback: Citizen scientists contribute data because they want to make a difference and learn something new (Bonney et al., 2009; Silvertown et al., 2013; van der Wal et al., 2016). Thus, training opportunities (either online or in person), and feedback (not simply in scientific journals) can be invaluable ways of retaining and attracting citizen scientists across the globe. Face-to-face training will also provide the opportunity to explain the value in submitting all data, including the reporting of small and/or unidentifiable carcasses.

(4) Vary the tools: Not all potential citizen scientists are likely to prefer the same method of collecting data. Thus, some individuals will be engaged by an interactive website (Swanson et al., 2015, 2016), while others prefer a smartphone app, and another group may prefer email communication (as was the case in this study).

Despite the limitations associated with reporting efforts, the EWT's citizen science project has established the first national database for animal road mortalities. This will guide future management decisions on mitigating the negative impacts of roads and provide a platform from which future studies can be designed.

AUTHOR CONTRIBUTIONS

We acknowledge the following for their contributions toward this manuscript: Data centralization and cleaning: WC and LR; Data analysis: SP; Manuscript writing: SP, LR, WC, and AIR.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00015/full#supplementary-material

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frontiers in Ecology and Evolution

REVIEW
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Wildlife Warning Reflectors' Potential to Mitigate Wildlife-Vehicle Collisions—A Review on the Evaluation Methods

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Wildlife-vehicle collisions (WVC) produce considerable costs in road traffic due to human fatalities as well as ecological and economic losses. Multiple mitigation measures have been developed over the past decades to separate traffic and wildlife, to warn humans, or to prevent wildlife from entering roads. Among these, wildlife warning reflectors (WWR) have been frequently implemented, although their effectiveness remains a subject of discussion due to conflicting study results. Here we present a literature review on the effectiveness of WWR for $N = 76$ studies, including their methodological differences, such as the type of WWR (model and color), study conditions, and study designs. We used boosted regression trees to analyse WVC-data addressed in the literature to compare WWR effectiveness depending on the study design, study conditions, effective study duration, length of the tested sections, time period of the study, data source, reflector type, and animal species. Our analyses revealed no clear evidence for the effectiveness of WWR in preventing WVC. Instead, our meta-analysis showed that most studies indicating significant effects of WWR on the occurrence of WVC may be biased due to insufficiencies in study design and/or the approach of WVC data acquisition. Our computation of log response ratios (LRR_{WVC}) showed that only studies applying a before-after (BA) design concluded that WWR were effective. Moreover, BRT modeling revealed that only studies of <12 months effective study duration and <5 km test site length indicated that WWR might lower WVC. Based on the vulnerability to confounding factors of WWR-study designs applied in the past, this review suggests the standardization of study conditions, including a before-after control-impact (BACI) or a cross-over study design with spatial and temporal control sections, a minimum test site length and a minimum study duration.

Keywords: animal-vehicle collisions, deer-vehicle collisions, wildlife mirrors, roadside reflectors, deer mirrors, swareflex, strieter lite, van de ree

INTRODUCTION

Since the beginning of the automobile era, wildlife-vehicle collisions (WVC) have strongly influenced the environmental impact of road traffic and have increasingly threatened both humans and wildlife (Stoner, 1925). Reliable data on economic and ecological costs is available to date for only a few European countries over the past three decades (cf. Langbein et al., 2011). For the year

1996 alone, in Europe 500,000 collisions with ungulates, 300 human fatalities and an economic loss of ca. one billion US dollars were estimated by Bruinderink and Hazebroek (1996). Two decades later, 263,000 officially reported WVC and an economic loss of almost 0.7 billion Euros were reported for Germany alone (GDV, 2017)[1]. The total damage in Europe overall can therefore be assumed to be far larger than in 1996. At present, a total of 800,000 WVC with ungulates is estimated for Germany, given that likely more than two-thirds of all collisions remain unreported, as reported for the US and Canada (Huijser and Kociolek, 2008; Snow et al., 2015; Hesse and Rea, 2016). However, WVC are not randomly distributed, but tend to accumulate in certain areas as a result of spatial and temporal factors (Gunson et al., 2011; Bíl et al., 2013). The duration of temporary WVC-hotspots is determined by diurnal and seasonal changes depending on species and climate conditions (Madsen et al., 2002; Compare et al., 2007). Furthermore, local differences in WVC-hotspots usually depend on species' habitat characteristics (Malo et al., 2004), type of road, and traffic volume (Clarke et al., 1998; van Langevelde and Jaarsma, 2009; Langbein et al., 2011; Beben, 2012). As mammals utilize landscapes at a different spatial scale than, for example, amphibians, predicting exact WVC-hotspots for these species is difficult (van Gelder, 1973; Ashley and Robinson, 1996; Madsen et al., 1998). Deer-vehicle collisions are the most common type of reported WVC in northern Europe (DeNicola et al., 2000; Rutberg and Naugle, 2008) and involve ∼6% of the roe deer (*Capreolus capreolus*) spring population (Bruinderink and Hazebroek, 1996); or up to 264,000 animals in Germany every year (GDV, 2017)[2]. WVC-hotspots and temporal aggregations for this species are potentially due to habitat structure (Finder et al., 1999; Nielsen et al., 2003; McShea et al., 2008), seasonality (Hubbard et al., 2000), and perhaps lunar cycles (Steiner et al., 2014; Colino-Rabanal et al., 2018). Although WVC depend on both human and deer activities (Mysterud et al., 2004), deer-vehicle collisions have increased by 25% over the past decade, whereas all non-deer-vehicle collisions have decreased by 5–10% (Hothorn et al., 2015). The overall increase in WVC may therefore be due to an increase in deer population density rather than an increase in human activity or traffic intensity.

Mitigation measures (cf. Iuell et al., 2003; van der Ree et al., 2015) to reduce WVC on roads are often accompanied by high costs for construction and maintenance, including fencing, green bridges, or electric warning signs (Kruidering et al., 2005; Huijser et al., 2007a). Other, less costly measures (e.g., olfactory repellents, wildlife warning signs, speed limit reductions, or specific training to warn humans) have been shown to be ineffective in the long term, partly due to habituation (Elmeros et al., 2011; Beben, 2012). So far, only optical alarm devices, such as wildlife warning reflectors (WWR) have been occasionally reported to reduce WVC, but their effectiveness remains in question, as findings are mixed and concomitant conclusions are highly contradictory (cf. Brieger et al., 2016). Previous reviews have surveyed outcomes on the effectiveness of WWR and have sometimes conducted meta-analyses to include national and international published studies (D'Angelo and van der Ree, 2015: $N = 13$ studies; Brieger et al., 2016: $N = 23$ directly available studies, $N = 18$ indirectly available studies, $N = 12$ newspaper articles and $N = 37$ not accessible studies). However, we identified a considerable number of additional peer-reviewed studies which have not been evaluated, and which also focus on the effectiveness of WWR (Supplementary Table 1).

In this review we provide an extensive summary of research findings on the effectiveness of WWR ($N = 65$ directly available studies, $N = 13$ indirectly available studies); and excluded non-scientific public articles as sources. As far as we know, this is to date the most comprehensive review on the effectiveness of WWR, with almost twice as many studies than the next comprehensive review [cf. (D'Angelo and van der Ree, 2015) ($N = 13$ studies), and (Brieger et al., 2016) ($N = 41$ studies, 12 newspaper articles)]. In addition, we focused on methodological differences due to the variability in WWR models, such as manufacturer, reflector color, as well as study approaches, such as study designs and collision reports. This is the first study testing WWR, of which we are aware, that examines the relationship between study approaches and study results. We also aimed to identify minimal requirements for a successful study design in order to make further recommendations for effective studies on WWR efficiency. Consequently, we tested the hypotheses that: (H1) existing study results can be explained by the specifics of study designs, and (H2) a meta-analysis of previous studies identifies minimal requirements for a successful study design.

FUNDAMENTALS

Wildlife Warning Reflectors

Optical warning devices, such as WWR, are mounted along the road on guideposts oriented toward the road verge. WWR are intended to prevent wildlife from entering a road when a vehicle passes at night, its headlights reflecting off the WWR toward the road verge. The reflections from several WWR are supposed to create a "fence of light" in front of animals in close proximity to the moving vehicles. This is believed to alter the behavior of animals and interrupt their movement toward the road (e.g., Beilharz, 2017[3]; Schilderwerk Beutha, 2017). WWR have been distributed since the early 1960s and are now available in diverse construction types and in a variety of colors. Among the first models were the "Van de Ree" mirrors, developed in the Netherlands (McLain, 1964; Nettels, 1965), followed by the "Ruppert" reflectors (Queal, 1968). More commonly applied and tested are the models "Swareflex," developed by Swarovski in 1973 in Austria (Rudelstorfer and Schwab, 1975) and "Strieter Lite," developed by Strieter Corp. in 1994 in the United States (Barlow, 1997). Other WWR were developed by Bosch and GFT (Bosch, "WEGU," and "AWIWA" reflectors), both in Germany (Gladfelter, 1984; Ujvari et al., 1998).

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[1] http://www.gdv.de/2016/11/wildunfaelle-erreichen-hoechststand-alle-zwei-minuten-kracht-es/. *Accessed* 08.02.2017.

[2] http://www.gdv.de/2016/11/wildunfaelle-erreichen-hoechststand-alle-zwei-minuten-kracht-es/. *Accessed* 08.02.2017.

[3] http://www.beilharz.eu/de/wildwarnreflektor.html. Accessed 08.02.2017.

Color Vision

WWR are most commonly produced in red, but also in white or amber colors (D'Angelo et al., 2006). Whereas humans are trichromatic and perceive red as a warning signal (Goldstein, 1942; Elliot et al., 2007), most mammals, including ungulates, are dichromatic with a high density of rods (Witzel et al., 1978; Jacobs et al., 1994, 1998). Thus, ungulates have one photo-pigment associated with a cone mechanism for short wavelengths with a peak between 450 and 460 nm (S-cone), and a second photo-pigment associated with a cone mechanism 167 for middle wavelengths with a peak of 537 nm (M/L-cone) (e.g., Carroll et al., 2001). Therefore, red light with a wavelength of 650 nm exceeds the visible range of ungulates (Jacobs et al., 1994; Yokoyama and Radlwimmer, 1998; Pürstl, 2006). Thus, recently developed WWR models have been adjusted accordingly, and are now produced in colors of shorter wavelengths, such as green and blue (e.g., Beilharz, 2017[4]; Brieger et al., 2017a,b; Kämmerle et al., 2017; Schilderwerk Beutha, 2017).

MATERIALS AND METHODS

Literature Survey and Study Selection

The available literature was surveyed systematically using the online databases ISI Web of Knowledge (webofknowledge.com) and Google Scholar (scholar.google.de). The search was conducted by combining the terms ("wildlife" OR "deer" OR "roadside" OR "animals") AND ("reflectors" OR "mirrors") using multiple languages (Dutch, Danish, German, Norwegian, Swedish, and Spanish). We additionally tested the names of various manufacturers of WWR (cf. Supplementary Table 1). All studies (including empirical studies and reviews) were filtered for their relevance regarding the effectiveness of WWR. Subsequently, we surveyed the reference lists of relevant studies for additional older studies which had not been recorded. In total, we found 76 publications evaluating the effectiveness of WWR between 1964 and 2017 (cf. Supplementary Table 1). Twelve of these studies were not accessible, but relevant information is presented indirectly through later studies in which they were cited.

Data Extraction and Data Processing

Each study was scanned for information on the reflector, reflector color, and manufacturer. If available, the respective species was documented and classified as cervid species, marsupial species, or other. All information on study duration (length in months), study location (e.g., field, enclosure, or laboratory) as well as the number and length in road distance of test- and control sites was listed. Additionally, we captured the effective study duration, which quantifies the effective duration of a test or control period, including or precluding the use of reflectors, respectively. Furthermore, the applied study design was identified [e.g., before-after (BA), control-impact (CI), before-after control-impact (BACI), cover/uncover(C/U), behavior, other) and, finally, the number of WVC was documented. We also collected information on the data source for counts of WVC (e.g., police, transportation administrations, research group or hunters) and the statistics used for analyzing the data (e.g., *t*-test, chi-square). To make possible an adequate comparison of observed occurrences between studies, we normalized each count of WVC to 1 year each of the effective study duration (time period of a test or control measurement) and 1 km of road distance ($\mathrm{WVC}_{\mathrm{norm}}$, $\mathrm{year}^{-1}\ \mathrm{km}^{-1}$). In total, 41 sets of WVC data with and without reflectors were considered for our analysis (Supplementary Table 1). Using $\mathrm{WVC}_{\mathrm{norm}}$, we calculated the log response ratio (LRR) as an effect size measure of WWR-effectiveness, thus quantifying the effect of the mean outcome in the experimental group (i.e., with WWR) in comparison to the control (i.e., without WWR) as described by Hedges et al. (1999).The LRR represents a suitable metric for meta-analysis of count data, which can be easily compiled without knowledge of data variances and sample sizes of single studies (Borenstein et al., 2009).

Statistical Analysis

Statistical analyses were performed using the R system for statistical computing (R Core Team, 2018, version 3.4.3). The response variables $\mathrm{WVC}_{\mathrm{norm}}$ and $\mathrm{LRR}_{\mathrm{WVC}}$ were tested for normal distribution and homoscedasticity of variances (Zuur et al., 2010; Fox, 2015). Depending on the data structure of $\mathrm{WVC}_{\mathrm{norm}}$, we applied parametric (paired student's *t*-test) or non-parametric statistics (Mann-Whitney U-test) to test for mean differences between each test- and control- group (WWR vs. no WWR). In the case of $\mathrm{LRR}_{\mathrm{WVC},}$ we used a one-sample *t*-test to analyze whether the mean effect size was different from zero. Each time multiple comparisons were conducted we additionally implemented a Bonferroni correction. To model the significance of the study design and site conditions on the effect size of WWR, we applied a boosted regression tree (BRT) analysis. This machine learning procedure combines the regression tree approach (De'ath and Fabricius, 2000) with a boosting procedure aimed at achieving optimized model accuracy (Schapire, 2003). BRT analysis is suitable for the interpretation of ecological data as it can combine analysis of nominally and metrically-scaled data, and due to its robustness with respect to unbalanced designs, can accommodate missing data and implement interaction effects of independent variables. The interpretation of the model output is straightforward since the relative importance as well as fitted functions for each predictor variable in use can be computed (Elith et al., 2008). For this analysis we used the R package *gbm* in combination with BRT function *gbm.step*() as developed by Elith et al. (2008).We aimed to explain the variance in $\mathrm{LRR}_{\mathrm{WVC}}$ and we therefore tested the importance of various possible predictors: (i) study design (BA, CI, BACI, CU); (ii) test road distance; (iii) effective study duration; (iv) data source for counts of WVC (authorities, hunters, scientists, others); (v) age of publication (1970s, '80s, '90s, 2000s, '10s); (vi) study region (North America, Europe, Australia); (vii) type of wildlife (cervids, marsupials, others); (viii) reflector type (Strieter, Swareflex, etc.); and (ix), reflector color (red, white, etc.,). Since the number of observations was too small to run a BRT model testing the importance of all possible predictors simultaneously [$N = 9$, reflector type, reflector color, study design, species, length of testing sites, study period, data source, effective study duration

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[4] http://www.beilharz.eu/de/wildwarnreflektor.html. Accessed 08.02.2017

(i.e., times reflectors are "active") and study region], we applied a core model using study design, effective study duration and test distance as permanent predictors. To additionally select the most influential predictors from these three, we implemented a series of BRT models, in each case adding the two other possible predictors in all possible combinations. Variables were considered as predictors for the final model only when their relative importance was not below 5%. The final model was fitted as 10-fold replication and the results were averaged to present a mean outcome of the partially stochastic procedure.

RESULTS

Behavioral, Physiological, and Spectrometric Studies on the Effectiveness of WWR

Behavioral studies of the reactions of animals to WWR ($N = 10$) did not show any effect that would lower WVC or any reaction of animals that would decrease the risk of WVC. The reactions of different deer or marsupial species were examined mainly for Swareflex and Strieter Lite Warning Reflectors (e.g., Griffis, 1984; Zacks, 1985; D'Angelo et al., 2006; Ramp and Croft, 2006). No study found any flight behavior or increased vigilance of animals when WWR or other light sources were activated (e.g., Sheridan, 1991; Norman, 2001). If anything, D'Angelo et al. (2006) showed that deer were more likely to be involved in negative deer-vehicle interactions, i.e. that the chance of a collision between deer and approaching vehicles increased, when WWR were installed than in periods without reflectors. Moreover, spectrometric analyses of WWR showed that the reflected light intensity was infinitesimal even at short distances from the reflectors and was additionally diminished by the headlights of approaching vehicles (Sivic and Sielecki, 2001; Schulze and Polster, 2017).

Methodological Differences and Results of WWR Studies

In total, we found 76 publications evaluating the effectiveness of WWR between 1964 and 2017 (62 directly and 14 indirectly accessible, cf. Supplementary Table 1). Most studies ($N = 51$) conducted analyses of WWR in the field using either a before-after ($N = 29$), control-impact ($N = 5$), BACI ($N = 8$), or cover/uncover ($N = 10$) study design. Of these, 39 studies provided 41 data sets which could be standardized to WVC year^{-1} km^{-1} with and without reflectors. Information on study duration and road length of the study sites was available in 42 and 43 studies, respectively. Behavioral analyses of wildlife and WWR were conducted in 10 studies (e.g., Ujvari et al., 1998). Additionally, four studies analyzed optical response measures of cervids with respect to WWR effectiveness, but reflectors were not tested directly in these studies (Almkvist et al., 1980; Zacks and Budde, 1983; Martschuk, 2014; Brieger et al., 2017b), thus these studies were not considered further. Other studies used spectrometric ($N = 2$), physiological ($N = 1$), or meta- ($N = 1$) analyses to evaluate the efficacy of WWR (cf. Supplementary Table 1). Twenty studies concluded that WWR reduce WVC and 18 studies found no effect or no conclusion was provided (**Figure 1**), while only 15 datasets showed a decline in WVC. Moreover, 26 data sets demonstrated (and 38 studies concluded) that there was an increase in WVC after WWR implementation.

Wildlife warning reflector models evaluated in the literature were mainly Swareflex reflectors ($N = 39$). A slightly different model (Sivic and Sielecki, 2001), the Strieter Lite WWR, was tested in 16 studies (e.g., Barlow, 1997; Riginos et al., 2015, 2018). Other reflectors evaluated were WEGU ($N = 2$, e.g., Olbrich, 1984), AWIWA ($N = 2$, e.g., Voß, 2007), Bosch ($N = 2$, e.g., Gladfelter, 1984), Ruppert ($N = 1$, Queal, 1968), ITEK ($N = 1$, van den Berk, 2017), and Beutha reflectors ($N = 3$, Pluntke, 2014; Brieger et al., 2017a; Kämmerle et al., 2017) (cf. Supplementary Table 1). Study duration testing the effectiveness of WWR varied from 0.75 months (Ujvari et al., 1998) to 300 months (Sielecki, 2001), depending on the study approach (e.g., behavioral observations of Ujvari et al., 1998 compared to a before-after study design of Sielecki, 2001; cf. Supplementary Table 1). In summary, the majority of authors concluded that WWR were either ineffective ($N = 19$) or even (marginally) increasing WVC with WWR ($N = 26$). Other authors resumed that an effect remained undetected ($N = 7$). In contrast, twenty studies indicated a decreasing trend in WVC with WWR (**Figure 1**, Supplementary Table 1). All the studies differed greatly in their methodologies (**Table 1**). It is notable that statistical analyses comparing WVC with and without reflectors applying a before-after design led to a significant reduction in WVC after implementation of reflectors ($p < 0.05$). Other study approaches revealed a tendency toward increases in WVC (e.g., behavioral studies, BACI, cover/uncover, **Figure 1**) or at least no reductions in WVC after installation of WWR (**Figure 2**). Only 14 publications that included information on WVC year^{-1} km^{-1} concluded that WWR reduce WVC ($N = 13$ before-after, $N = 1$ control-impact) (cf. Supplementary Table 1).

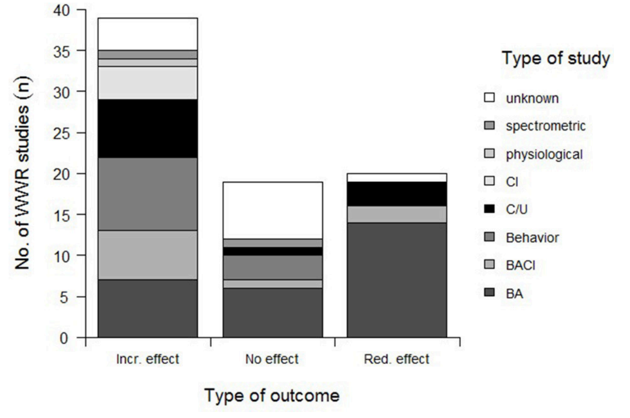

FIGURE 1 | Findings of the effectiveness of Wildlife Warning Reflectors (WWR) to reduce Wildlife-Vehicle collisions (WVC). Study designs included before-after (BA), control-impact (CI), before-after-control-impact (BACI), or cover/uncover (C/U) study approaches, as well as behavioral, physiological, and spectrometric evaluations. Unknown study designs ($N = 12$) did not provide information on the applied design or the information was not accessible.

TABLE 1 | Studies evaluating the effectiveness of wildlife warning reflectors (WWR) to reduce wildlife-vehicle collisions (WVC) on roads.

Study design	Statement	References
Before-after	–	Howe, 1967; Rudelstorfer and Schwab, 1975; Lück, 1977; Johnson, 1981; Paxson, 1981; Olbrich, 1984; Ingebrigtsen and Ludwig, 1986; Hildebrand and Hodgson, 1995; Pafko and Kovach, 1996; Schwabe et al., 2000; Stoyan, 2000; Grenier, 2002; Steiner, 2011; van den Berk, 2017
	*	Nettels, 1965; Beauchamp, 1970; O'Rourke, 1990; Johnson et al., 1993; Jared, 2002; Voß, 2007; Christensen, 2016
	+	McLain, 1964; Ladstätter, 1974; Dalton and Stranger, 1990; Waring et al., 1991; Sielecki, 2001; Binetruy and Kenny, 2005; Pluntke, 2014; Riginos et al., 2015
Control-impact	+	Ekblom, 1979; Gilbert, 1982; Reeve and Anderson, 1993; Cottrell, 2003; Bertwistle, 2009
Before-after control-impact	–	Lehtimaki, 1979; Gladfelter, 1984
	*	Boyd, 1966
	+	Queal, 1968; Gordon, 1969; Aspinall, 1994; Rogers and Premo, 2004; Gulen et al., 2006
Cover/uncover	–	Schafer et al., 1985; Fjeld and Antonsen, 1999; Lien Aune, 2004
	*	Barlow, 1997; Riginos et al., 2015
	+	Armstrong, 1992; Ossinger and Schafer, 1992; Ford and Villa, 1993; Libjå and Gundersen, 2001; Woodard et al., 1973; Woodham, 1991; Reeve and Anderson, 1993
Behavior	*	Konings, 1986
	+	Griffis, 1984; Zacks, 1985, 1986; Sheridan, 1991; Ujvari et al., 1998; D'Angelo et al., 2006; Ramp and Croft, 2006; Brieger et al., 2017a; Kämmerle et al., 2017
Physiology	+	Norman, 2001
Spectrometry	+	Sivic and Sielecki, 2001; Schulze and Polster, 2017
No information	*	Müller, 1977; Williamson, 1980; White, 1983; Kofler, 1984; Janssen and Claus, 1996; Pepper et al., 1998
	+	Garver, 1976; Mah, 1989; Scholten et al., 1989; Hester, 1991; Pepper, 1999

Studies are arranged by study design (i.e., before-after, control-impact, before-after control-impact, cover/uncover, behavior, physiology, spectrometry and without information on the study design). Studies were further arranged by the statement of the author on the effectiveness of the reflectors to lower WVC (–), increase WVC (+) or with no conclusion provided or found (*).

Effects of Study Characteristics on the Outcome of WWR Efficiency

Based on 41 datasets presented in 39 studies, a quantitative analysis of the effectiveness of WWR on mitigation of WVC and its dependence on study conditions was applied. With respect to BRT modeling, the pre-selection of predictors revealed insignificance of the variables *study region* (mean relative importance: 0.1%), *reflector type* (1.9%), *reflector color* (0.0%), and the considered *species* (0.0%). Accordingly, the final model included five predictor variables and explained, on average, 23.2% of the variance observed in $\mathrm{LRR}_{\mathrm{WVC}}$ (**Figure 3**). *Study design* was identified as the most influential predictor (mean relative importance: 32.7%) and the BRT revealed considerable differences between fitted values for the class *before-after* design in comparison to other study designs (**Figure 3**). The *time of the study release* as well as the *testing site length* of tested road segments indicated their relative importance for the accurate prediction of $\mathrm{LRR}_{\mathrm{WVC}}$ observed (22.1% and 23.1%, respectively; **Figure 3**). Fitted values of $\mathrm{LRR}_{\mathrm{WVC}}$ were generally higher using data from earlier published studies (1970–1990) and lower for more recent studies. With respect to *effective testing site length*, the fitted function showed a peak for the short road lengths studies (below 5 km) but the explanatory power was inconclusive for distances $> \sim 15$ km (**Figure 3**). Finally, the factor *data source* of WVC and *effective study duration* explained marginal degrees of the observed variance (relative importance: 14.0 and 8.2%,

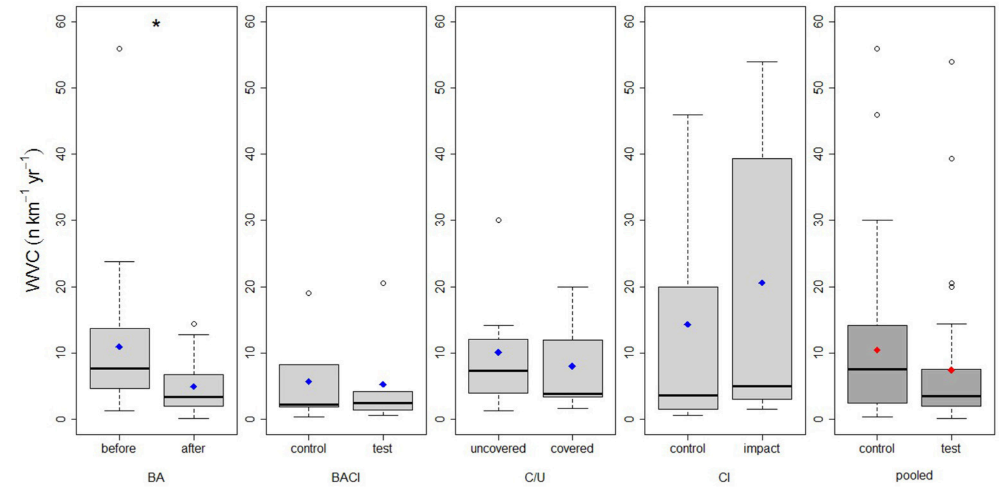

FIGURE 2 | Boxplots showing the influence of Wildlife Warning Reflectors (WWR) on Wildlife-Vehicle collisions (WVC) with respect to the study design. Data was standardized to WVC year^{-1} km^{-1} for both with and without reflectors. Studies performing a before-after (BA) comparison showed a significant reduction in WVC-data ($p < 0.05$). Before-after control-impact (BACI), covered/uncovered studies (C/U), as well as control-impact (CI) studies, did not show any significant effect of WWR on WVC-data. All standardized data comparing WVC year^{-1} km^{-1} for both, with and without reflectors WVC did not show any significant effect of WWR on WVC-data (pooled).

respectively). Fitted values of LRR_{WVC} varied slightly between datasets; for instance, those obtained from authorities, or data collected directly by scientists. The fitted function given for study duration followed the same curve as that of the road distance curve, with a high value plateau for low study durations (below 12 months) and a neutral prediction value above 20 months (**Figure 3**). Our statistical analyses, including BRT modeling, identified *before-after* study design, *effective study duration* <12 months and *effective testing sites length* <5 km as most influential variables on the tested "effectiveness" of reflectors.

DISCUSSION

The results of both the review and the analysis of WVC-data from literature indicated that the effectiveness of WWR remains questionable and that the observed effect of WWR on WVC largely depended on other factors such as study design, effective study duration, and effective testing site length.

According to our meta-analyses the reflector model (1.9%) or the color of the reflectors (0.0%) did not indicate any influence on WWR. However, the risk of WVC varies during the year and the time of day, with high risks during the rutting season, as well as in the morning and first hours of the night (Hothorn et al., 2015). As ungulates, such as roe deer, prefer open areas and agricultural fields during the night (Mysterud et al., 1999a,b), the frequency of road crossings increases during darkness (Hothorn et al., 2015). Therefore, scotopic and mesopic vision play an important role in the life of ungulates with diurnal patterns (Hanggi et al., 2007), concomitant with greater rod density and better light perception in the range of blue and blue-green (Szél et al., 1996; VerCauteren and Pipas, 2003). From this perspective, the value of long-wavelength WWR is questionable and the likelihood of a reduction of WVC can be argued given the lack of animals' ability to perceive colors in these wavelengths (VerCauteren and Pipas, 2003). Modern WWR, produced and marketed in the past decade, are primarily blue (e.g., Brieger et al., 2017a; Beilharz, 2017[5]; Kämmerle et al., 2017; Schilderwerk Beutha, 2017). However, independent studies evaluating the effectiveness of modern WWR in the field as well as the influence of blue light on feeding behavior in roe deer have not found any effect of the reflectors, either in reducing WVC directly or resulting in aversion or increased vigilance in roe deer (Brieger et al., 2017a,b; Kämmerle et al., 2017). Moreover, spectrometric analyses of WWR models have shown that the reflected light intensity is already very low at distances near the devices (Sivic and Sielecki, 2001) and reflector intensity is further overlaid by the headlights of approaching vehicles (Schulze and Polster, 2017). This applies especially to colored WWR (Sivic and Sielecki, 2001). Thus, it is doubtful that the light reflected from WWR has a sufficient intensity to elicit any reaction in animals at all.

Interestingly, some studies as well as observations by local hunters report their positive experiences with various models of WWR, including red models. A temporary reduction in WVC after installation of WWR may be explained by chance or by naturally oscillating fluctuations in population densities related, e.g., to hunting effort and food supply (Fryxell et al., 1991, 2010). Animals may also react aversively to something "new" in their environment (i.e., "novel object," cf. Forkman et al., 2007), so their reaction could be simply to the presence of the posts on which reflectors are mounted. In this case, the color of the reflector would not matter. Riginos et al. (2018) reported that carcass rates decreased by 33% when

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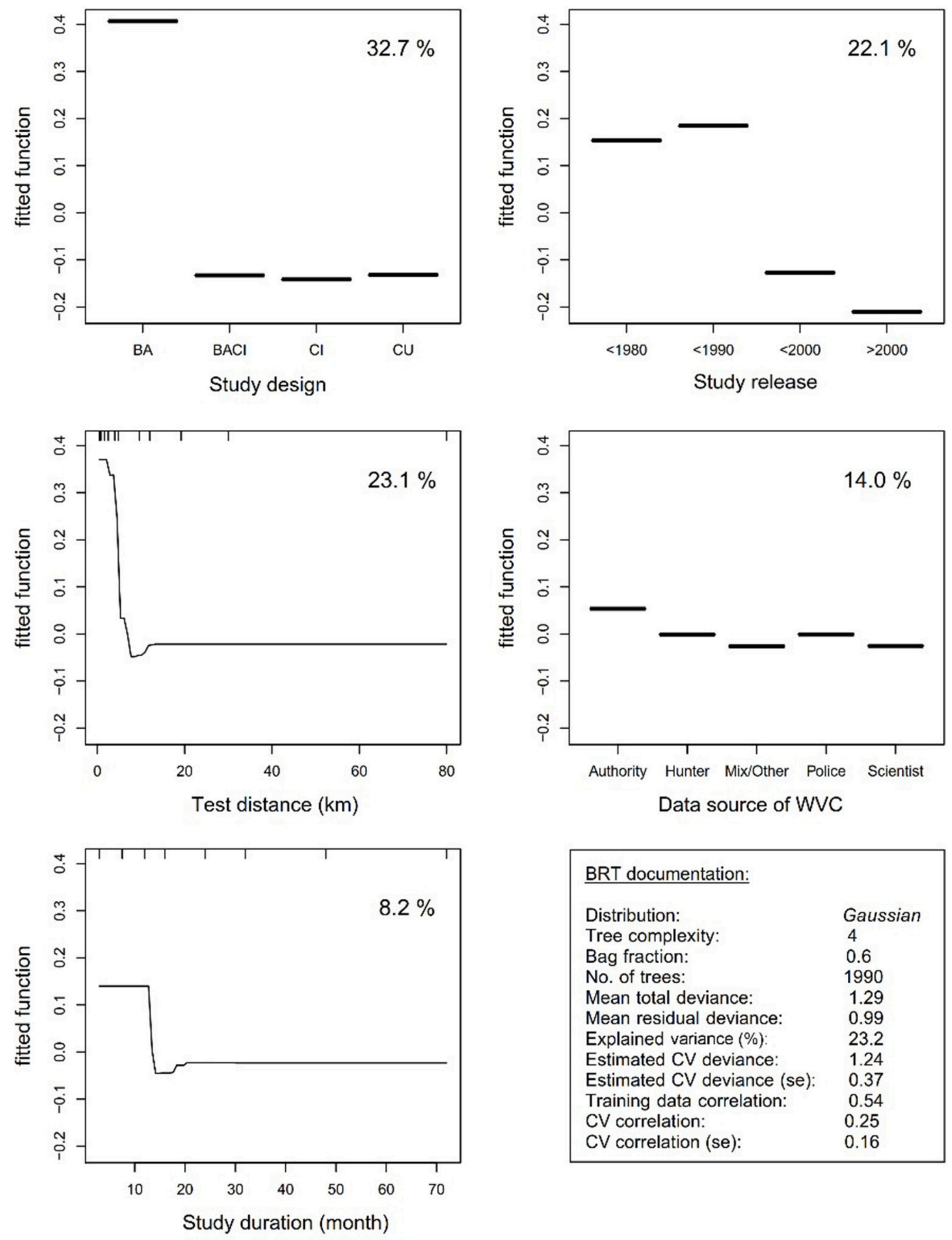
[5] http://www.beilharz.eu/de/wildwarnreflektor.html. Accessed 08.02.2017



BRT documentation:

Distribution:	*Gaussian*
Tree complexity:	4
Bag fraction:	0.6
No. of trees:	1990
Mean total deviance:	1.29
Mean residual deviance:	0.99
Explained variance (%):	23.2
Estimated CV deviance:	1.24
Estimated CV deviance (se):	0.37
Training data correlation:	0.54
CV correlation:	0.25
CV correlation (se):	0.16

FIGURE 3 | Partial response plots of the five explanatory variables in the boosted regression tree (BRT) model including BRT documentation, such as explanatory variance of the model (23.2%), and values indicating the relative influence of the explained variance in the BRT model, for each variable respectively. Study designs included before-after (BA), before-after control-impact (BACI), control-impact (CI), and cover/uncover (CU) approaches. Data source was separated by authority from which data were obtained, i.e., transportation administration, road authorities), hunters, mix/other (i.e., more than one data source was used), police (i.e., WVC which were officially reported to the local police station) and scientists.

delineator posts were covered with white canvas bags compared to uncovered reflectors, but carcass rates were 32% lower with uncovered reflectors than with posts covered with black canvas bags. Thus, white canvas and reflectors might stand out more from the surrounding landscape than black. However, the animals could be expected to become habituated to the presence of these objects over time, with a resulting decline in their effectiveness. Reduction in WVC may also be due to the influence of the reflectors on the behavior of drivers rather than on the behavior of animals (Zacks, 1985), as the light intensity from the direct reflection to the driver is far larger than the reflection to the surroundings of the road (Schulze and Polster, 2017). An increased attention of drivers to wildlife near the road has been reported for studies testing deer-whistles, resulting in decreasing WVC (Zacks, pers. comm. 2015). However, the response of drivers to WWR has not been evaluated. It is also possible that the reflectors serve as a warning device that influences driver behavior (Rowden et al., 2008), but habituation might be expected as has been shown for wildlife warning signs (Huijser et al., 2007b).

BRT modeling showed that especially short studies, with <12 months of effective study duration and <5 km test sites and a before-after approach, showed a decrease in WVC with WWR. Thus, we could confirm our hypotheses that (H1) study results can rather be explained by the specifics of study designs than by the presence of WWR. Additionally, we can partly confirm our second hypothesis (H2) that examination of previous studies made it possible to infer minimal requirements for a successful study design: before-after study design, effective study duration <12 months and effective testing sites length <5 km as the most influential variables on the tested "effectiveness" of reflectors.

Before-after study designs most often detected a decrease in WVC with WWR, but it is possible that control and testing periods may not have used the same season. Activity patterns of ungulates are reflected in WVC peaks, especially during the rutting season of each species (Allen and McCullough, 1976; Lavsund and Sandegren, 1991; Hothorn et al., 2015). There could be a decrease in animals' vigilance during this period. A WVC peak during dusk and dawn, especially in the darker seasons (Steiner et al., 2014), in which high traffic volumes—such as during rush hours—coincide with an increase in the activity phase of animals. Thus, studies including all activity periods of animals are more likely to include all variables (e.g., mating season or fawning) that may influence the reactions of wildlife to oncoming vehicles. Although Brieger et al. (2016) note that a before-after study design requires at least 8 years of study to gain solid data on the effectiveness of WWR to reduce WVC, a longer study duration could be confounded by environmental changes or population fluctuations over that time period and thus affect the outcome of studies testing the effectiveness of WWR (Fryxell et al., 2010; Brieger et al., 2016).

LRR_{WVC} analyses showed that only studies applying a before-after study design found a decrease in WVC with WWR. When studies that applied the before-after or control-impact designs were omitted, WWR did not lower WVC. Moreover, BRT models showed that the applied study design explains most of the variance ($>30\%$). Studying the impact of a single treatment in a paired study design usually takes the form of studying a population before and after a treatment or by studying two very similar populations or locations (Morrison et al., 2008). However, other potential factors influencing a change may complicate the interpretation of experiments, increasing the Type II error, as heterogeneity results in the confounding of experimental errors (Underwood, 1997; Morrison et al., 2008). Study designs such as before-after or control-impact designs lack the independence of different levels of single treatments and true replication (Morrison et al., 2008). Thus, although there may be no statistical problem with the study and the null hypotheses is rejected, a potential change after the implementation of a treatment cannot simply be assigned to that impact, but may be due to other factors such as weather, crop rotation, etc., (Underwood, 1997; Morrison et al., 2008). Therefore, results comparing the number of WVC before and after the implementation of WWR, as well as comparing test sites with control sites, must be treated with caution due to discontinuity in time or space. In these, BACI and cross-over study designs provide a remedy, as they have the highest inferential strength for assessing impacts on the environment (Green, 1979; Underwood and Chapman, 2003; Roedenbeck, 2007).

In addition to a number of influencing variables such as *reflector model*, *reflector color* or *effective testing site length* in road distance and *effective study duration*, the *type of data collection* also seems to affect the results of studies testing the effectiveness of WWR. BRT analyses showed that data sources influence the variance in the model by 15%. Also, the opinion of the authors can influence study results. While Gladfelter (1984) stated that WWR reduced the number of WVC significantly, he compared test and control sites that differed strongly in WVC numbers, challenging the control-impact approach. Moreover, WVC differed among test sites after installation of WWR; thus not all test sites showed a reduction in WVC after implementation. When his data were standardized, WVC changed only from 1.86 WVC $\text{year}^{-1}\ \text{km}^{-1}$ without WWR and 1.39 $\text{year}^{-1}\ \text{km}^{-1}$ with WWR (cf. Supplementary Table 1). Similar issues apply to the study conducted by Hildebrand and Hodgson (1995). While WVC were rather low before installation of WWR at two test ($N = 1$ WVC) and control sites ($N = 2$ WVC), numbers increased to 3 WVC year^{-1} at the test sites and 2.75 WVC year^{-1} at the control sites after installation. Standardizing this data to the test site length, WVC actually increased from 0.38 WVC $\text{year}^{-1}\ \text{km}^{-1}$ without reflectors to 0.6 WVC $\text{year}^{-1}\ \text{km}^{-1}$ with reflectors. With so few observations, a test for significance is not actually possible. However, the authors stated that they found a non-significant reduction in WVC comparing test and control sites, and concluded that WWR are effective in reducing WVC. Olbrich (1984) compared test sites that he maintained. Although WVC differed strongly among test sites after installation and no statistical test was applied, he concluded that WWR reduce WVC. In additional examples of previous study limitations, Pafko and Kovach (1996) compared data before and after installation of WWR, yet without proper information on WVC before the study and with an invalid type of data collection. However, the authors concluded that WWR effectively reduced WVC. Other studies have also failed to provide data on the numbers of WVC before installation of WWR (e.g., Nettels, 1965). For example Grenier (2002) conducted a meta-analysis including data from different highway and transportation agencies. He concluded that WWR are effective in reducing WVC, although it remains unclear which studies he considered, as studies without any effect of WWR were excluded.

CONCLUSIONS

The effectiveness of WWR remains doubtful, due to conflicting study results and questionable study designs, especially using the before-after approach. BRT modeling indicated that only studies with <12 months effective study duration and <5 km test sites found a decrease in WVC with WWR. Moreover, LRR_{WVC} analyses showed that only studies applying a before-after approach concluded that WWR was effective. This design however, lacks the independence that would accrue from different levels of single treatments and true replication (Morrison et al., 2008). Thus, a potential change after the implementation of a treatment cannot simply be assigned to that impact, but to other factors as well. (Underwood, 1997; Morrison et al., 2008). Additionally, analyses of physiological abilities and spectrometric requirements in the literature provide evidence that most mammals cannot effectively perceive red light and that reflected light has insufficient intensity to elicit any reaction in animals that would lead to a decreased risk of WVC. Thus, to include as many explanatory variables, but also to exclude as many confounding factors (environmental biases) as possible, a BACI or cross-over design (Roedenbeck, 2007; Morrison et al., 2008) is advisable. Furthermore, predictive variables such as test site length, effective study duration, and data source influenced the variance observed in LRR_{WVC}. Additionally, behavioral observations of animals reacting to WWR including all activity periods, especially WVC-peak seasons, are recommended for further studies testing the effectiveness of modern WWR.

AUTHOR CONTRIBUTIONS

AB: Idea for this publication, Literature Survey, Data Collection, Data Analyses, Statistics, Writing the manuscript; PA: Statistics, comments on the manuscript; TV: Project idea, Organization of funding, comments on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00037/full#supplementary-material

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Performance of Arch-Style Road Crossing Structures from Relative Movement Rates of Large Mammals

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In recent decades, an increasing number of highway construction and reconstruction projects have included mitigation measures aimed at reducing wildlife-vehicle collisions and maintaining habitat connectivity for wildlife. The most effective and robust measures include wildlife fences combined with wildlife underpasses and overpasses. The 39 wildlife crossing structures included along a 90 km stretch of US Highway 93 on the Flathead Indian Reservation in western Montana represent one of the most extensive of such projects. We measured movements of large mammal species at 15 elliptical arch-style wildlife underpasses and adjacent habitat between April and November 2015. We investigated if the movements of large mammals through the underpasses were similar to large mammal movements in the adjacent habitat. Across all structures, large mammals (all species combined) were more likely to move through the structures than pass at a random location in the surrounding habitat. At the species level, white-tailed deer (*Odocoileus* virginianus) and mule deer (*O. hemionus*) used the underpasses significantly more than could be expected based on their movement through the surrounding habitat. However, carnivorous species such as, black bear (*Ursus americanus*) and coyote (*Canis latrans*) moved through the underpasses in similar numbers compared to the surrounding habitat.

Keywords: road ecology, fragmentation, connectivity, mammal, highway, underpass, mitigation

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INTRODUCTION

Over the past few decades, a range of negative effects of transportation infrastructure on wildlife populations have been well documented (e.g., Forman and Alexander, 1998; Spellerberg, 1998; Trombulak and Frissell, 2000; Coffin, 2007). Roads and traffic affect wildlife populations through direct mortality from vehicle collisions and indirect effects associated with habitat loss and degradation. Linear infrastructure can also be a barrier to wildlife movement as animals may avoid open habitat with an unnatural surface and the disturbance associated with roads (e.g., moving vehicles, noise, and light; D'Amico et al., 2016). Avoidance of roads decreases connectivity and can threaten population viability and genetic variability (Wang and Schreiber, 2001). At over 14 million lane-kilometers of paved roads in the United States alone (USDOT, 2017) and a forecast of an additional 25 million lane-km globally by 2050 (Dulac, 2013), road networks will continue as one of the largest, most direct impacts humans have on ecosystems.

Wildlife-vehicle collisions and the barrier effect of roads are typically mitigated through wildlife fences in combination with wildlife crossing structures (Glista et al., 2009; Grilo et al., 2010; Kociolek et al., 2015). In order to continue to justify these measures, researchers must prove that these measures are effective in both reducing wildlife-vehicle collisions and providing connectivity for wildlife, then formulate suggestions on how to improve their performance.

The size and cost of wildlife crossing structures makes experimental design and testing in controlled settings difficult. Consequently, we have a poor understanding of the relative importance of structural attributes (design) and locational attributes (placement) to crossing performance. Furthermore, most mitigation projects that involve wildlife fences and wildlife crossing structures primarily address an immediate problem (e.g., reducing wildlife vehicle collisions) and are not intended to contribute novel data (Rytwinski et al., 2015). Wildlife mitigation measures are usually considered late in the project planning process after limitations on design or placement have already been imposed (Cramer and Bissonette, 2007; Kroll, 2015). Even for large projects, the number of crossing structures that have similar dimensions is generally too low for a quantitative analysis of their performance.

Many studies have documented absolute use of crossing structures by recording the number of animals that cross through a structure. However, absolute use alone provides little information on a structure's performance, as local wildlife population density and the specific configuration of landscape elements influence wildlife use of individual structures. To date, there are only very few studies that have assessed the effectiveness of crossing structures based on a rigorous comparison to animal abundance in the surrounding habitat (Clevenger and Waltho, 2005; but see van der Ree et al., 2007; van der Grift et al., 2013).

In this study, we evaluated the effectiveness of wildlife crossing structures associated with the highway US 93 North through the Flathead Indian Reservation in western Montana, USA. We measured performance of structures by comparing large mammal movements through the structures to those in the immediate surroundings. We were interested in investigating the performance of underpasses for animals that were willing to come close to the highway as crossing structures cannot be expected to serve animals that avoid transportation corridors altogether. Therefore, we located control plots directly adjacent to the road, within the road effect zone (Forman, 2000).

We first investigated the corridor-wide performance from 15 wildlife passage structures of similar geometry and design by contrasting wildlife movement through the structure to those in the surrounding habitat. Our analysis focuses on four target species including white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), black bear (*Ursus americanus*), and coyote (*Canis latrans*), and composite groups (deer sp., carnivore sp., and large mammal sp.). Secondly, we compared the crossing performance of these species for each individual structure. Additionally, we investigated if mitigation measures were concentrating or funneling wildlife through the crossing structures. Finally, we used the observed performance to estimate the percentage of total road length that would require crossing structures of the focal design in order to allow complete permeability for wildlife.

METHODS

Study Area

We collected data along US 93 in the Flathead Reservation between Evaro and Polson, Montana (**Figure 1**). The road runs north-south through the Flathead Valley, a heterogeneous landscape comprised of shrub, grassland, forests, wetland habitat, and agricultural lands. This section of highway received an annual average of 7,059 vehicles per day (MDT, 2014). The roadway design includes both undivided and divided, four-, three-, and two-lane highway accommodating a maximum speed limit of 70 mph (113 km/h), reducing to 25–45 mph (40–72 km/h) in towns.

Reconstruction of the 90.6 km section of highway began in 2004 to improve traffic flow and safety. The Confederated Salish Kootenai Tribes and the Federal Highway Administration agreed that the reconstructed highway must be respectful to the communities and people, but also to the land and wildlife that are considered both natural and cultural resources for the Tribes (Marshik et al., 2001; Kroll, 2015). Wildlife fences and crossing structures were an integral part of this "context-sensitive design" (Marshik et al., 2001).

The US 93 project is unique in the diversity of structure designs employed and in the number of replicates of select designs across a variety of habitat types. To date, the project includes 39 wildlife crossing structures of various designs from small concrete box culverts to a vegetated overpass and disjointed wildlife fencing (Huijser et al., 2016a).

Experimental Design

In this study, we address two primary questions: how does wildlife movement through crossing structures compare to wildlife movement in the immediate surrounding, and what is the effect of location, independent of structure design, on promoting wildlife passage. To investigate the first question, we used a control-impact study design (van der Grift et al., 2015). By selecting only congruent structures for the control-impact assessment, we were able to hold the effect of structure design constant and investigate only the effect of the structures' locations.

Selection of Wildlife Crossing Structures

While other studies have investigated the performance of structural attributes across multiple design types (e.g., Yanes et al., 1995; Clevenger and Waltho, 2000; Ng et al., 2004), this study is unique in selecting 15 structures of nearly identical design. These structures are the most common design included in the US 93 reconstruction (18 of 39) and they are used by a wide array of medium and large mammal species (Huijser et al., 2016a).

All structures are elliptical, corrugated metal arch-style underpasses with soil substrate, primarily built for large mammal passage. The structures have an average width of 7.32 m (range = 6.86–7.95 m), height of 5.55 m (range = 3.65–5.55 m), and length of 26.5 m (range = 14.6–40.0 m; **Table 1**). All structures

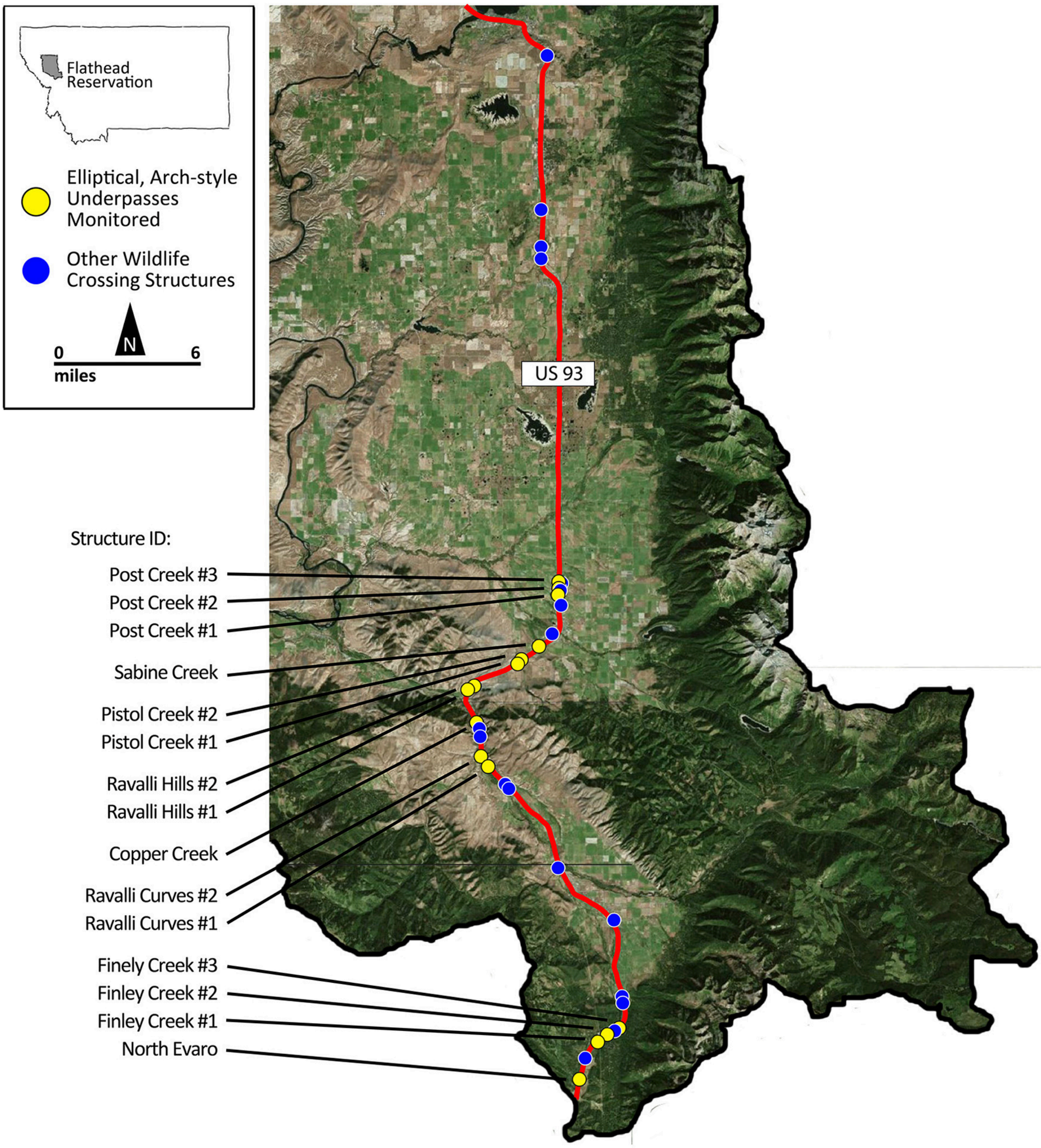

FIGURE 1 | The location of the wildlife crossing structures along US Highway 93 on the Flathead Indian Reservation in western Montana, USA. Structures monitored as part of this study are indicated as yellow circles. Other wildlife passage structures not included in this study are indicated as blue circles. US Highway 93 is shown as a red line. Base map from Google Earth (2016). Google Earth 7.1.7.2606. (2016). Flathead Indian Reservation, Flathead County, MT, USA 47.337894°N, 114.052525°W. Retrieved: 20 November 2016. Available at: http://www.google.com/earth/index.html.

include concrete retaining walls that extend out from the structure at an ~35° angle to the road and extend to ~10 m. Wildlife exclusion fencing (height 2.4 m) is associated with some structures (**Table 1**).

Monitoring Scheme and Sampling Methodology

We installed motion-sensing trail cameras (HyperFire PC900 Reconyx^{TM}; Holmen, WI) with infrared illumination to record wildlife. Two cameras were installed at each structure—one at each entrance. Ten additional cameras were installed at randomly generated locations within a 300 by 300 m control plot adjacent to each side of an underpass and the highway (five cameras on each side; **Figure 2**). We chose a control plot size of 300 m because this is the most restricted daily active movement radius of the most common species (White-tailed deer; Dusek et al., 1989). We assumed that animals willing to approach the road at this distance were likely to interact with the structure at a

TABLE 1 | Structural and locational attributes of the elliptical, corrugated metal wildlife crossing structures monitored in the study including dimensions, presence of associated wildlife exclusion fencing, distance to nearest crossing alternatives*, percent difference in development and cover in surrounding habitat on either side of the road**, and year of construction.

Site name	Site code	Width (m)	Height (m)	Length (m)	Fencing	Distance to nearest crossing alternative (m)	Δ% Cover	Δ% Developed	Year constructed
North Evaro	NEV	7.75	5.10	25.8	N	1	8.9	6.7	2010
Finley Creek #1	FC1	7.95	5.55	32.0	Y	1	61.1	22.0	2010
Finely Creek #2	FC2	7.95	5.55	21.9	Y	102	0.3	0.3	2010
Finely Creek #3	FC3	7.75	5.10	24.7	Y	298	6.8	6.8	2010
Ravalli Curves #1	RC1	6.86	4.78	22.0	Y	935	6.4	57.4	2006
Ravalli Curves #2	RC2	6.86	4.78	25.6	Y	1	36.3	7.3	2006
Copper Creek	COPC	7.75	5.10	18.3	Y	181	43.5	2.2	2006
Ravalli Hills #1	RH1	7.30	5.20	39.0	Y	434	0.0	26.9	2007
Ravalli Hills #2	RH2	7.30	5.20	31.2	Y	434	0.0	36.6	2007
Pistol Creek #1	PIC1	7.30	5.20	40.0	N	1	0.0	41.3	2007
Pistol Creek #2	PIC2	7.30	5.20	40.0	N	1	0.0	20.8	2007
Sabine Creek	SABC	7.32	3.65	14.6	Y	45	60.9	60.9	2007
Post Creek #1	POC1	7.32	4.75	28.8	Y	29	29.8	29.8	2007
Post Creek #2	POC2	7.32	4.75	22.0	Y	40	21.3	21.3	2007
Post Creek #3	POC3	7.32	3.90	19.5	Y	37	39.1	39.1	2007

**The distance to the nearest crossing opportunity was measured as: the distance to the next suitable crossing structure if fencing was continuous, distance to fence end if discontinuous, or 1 m if no fence was associated with the structure.*

***ArcGIS was used to calculate percent cover and habitat type from the National Land Cover Data for Montana (USGS, 2014). In calculating percent cover, land types: deciduous forest, evergreen forest, mixed forest, and woody wetlands were considered presence of cover and all others were considered absence of cover. Habitat type was assigned as a percentage distribution of natural and non-natural habitat. Land types: low intensity residential, high intensity residential, commercial/industrial/transportation, pasture/hay, and row crops were considered non-natural/developed and all others were considered natural/undeveloped.*

daily timescale. To simplify terminology, hereinafter we refer to the crossing structure simply as "structure" and refer to the 300 by 300 m control areas on each side of the crossing structure as "surroundings." We use the term "site" to refer to both the surroundings and structure at a given crossing structure location.

We chose to use the same sampling method to record wildlife movements at both the structure and surroundings in order to control for species-specific detection rates that contribute bias to studies using multiple detection methods (Ford et al., 2009). All cameras were set to capture a rapid succession of 10 images per trigger event. In order to standardize the observation range, we placed a stake 10 m from the camera. Only individual animals that crossed within 10 m and associated conspecific animals in a group were considered for analysis. The infrared illumination from the trail cameras is effective up to 15 m. By restricting our observations to 10 m we reduced the possibility of false negatives. The entrances to the structures are generally slightly narrower than 10 m. To maintain consistent measurements, we moved cameras at structures out from the entrance along the angled retaining wall until a 10 m viewing distance parallel to the road was reached (usually 1–2 m from the entrance). Remote monitoring by camera traps was carried out in accordance with the approval of University of Montana Instructional Animal Care and Use Committee.

We were not able to reliably identify individual animals from camera images; therefore, we treated an observation of a unique movement as our sampling unit. A movement was defined as any animal recorded within 10 m of the camera separated by at least 5 min from the next observation. Allen (2011) found that, in the same study area, for groups of the three most common species (white-tailed deer, mule deer, and black bear), either all or none of the individuals in a group crossed through underpasses. Because underpasses are flanked by retaining walls, all animals that enter must cross within the 10 m observational distance of the camera. Therefore, in order to compare observations between the structures and surroundings, for obvious conspecific groups in the surroundings, all animals in the group were recorded as individual movements if at least one animal in the group crossed within 10 m viewing window.

The 12 cameras at a site (10 in surroundings, 2 at the structure) remained in place for ∼2 weeks before we moved them to another site. The order in which sites were sampled was randomly assigned to reduce correlation between the effect of location and season. However, the order was also subject to our ability to obtain landowner permission to access the site. In cases where we could not immediately access a site, that site was skipped until permission could be obtained.

Analysis of Structure Performance

Total movements recorded in a 24 h period from cameras at a site were summed and divided by the number of cameras deployed at the structure or in the surroundings, respectively, to get observed and expected daily movement rates. Only cameras that operated for all sampling days at a site, and only full 24-h days were considered in analysis. Rates recorded in the surroundings and at the structure from the same 24 h period were compared to control for within-day temporal variability. Prior to statistical

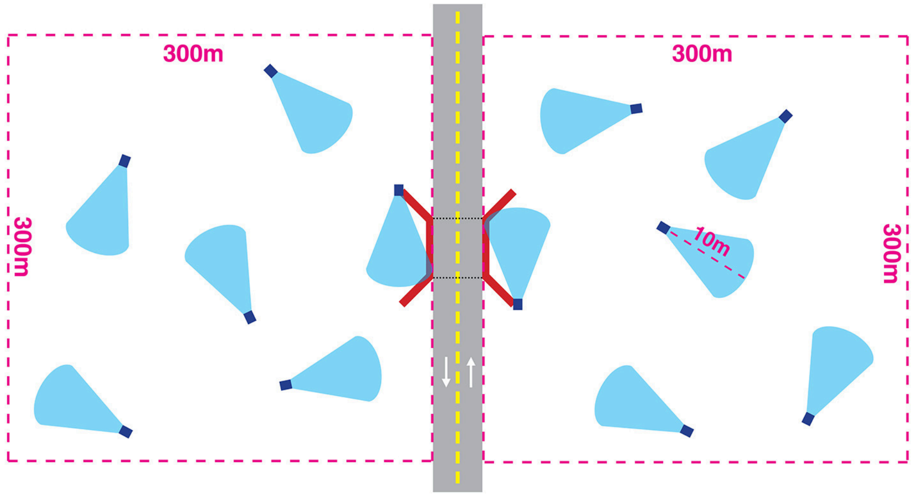

FIGURE 2 | Camera placements in relation to a wildlife highway crossing structure (not to scale). Twelve HyperFire PC900 Reconyx^{TM} trail cameras (dark blue squares indicate cameras, light blue cone indicates approximate 40° sampling window) were installed at each site for ∼2 weeks at each site. Ten cameras were installed at randomly generated points at least 50 m apart within a 300 m by 300 m area adjacent to each side of the structure (red lines represent concrete retaining walls associated with all crossing structures). Two cameras were installed at each entrance to the structure. Cameras were installed ∼3 m from the ground and a stake was placed to demarcate a 10 m viewing distance commensurate with the viewing distance of the cameras at the structure entrance.

analysis, we tested daily performance measures for temporal autocorrelation between days, finding none.

In order to assess a structure's effectiveness, we calculated performance differentials for each structure, broken down into species and species groups. We also calculated an overall effectiveness of all structures collectively for each species and species group. To calculate performance differentials, expected crossing rates from the surroundings were subtracted from observed crossing rates at the structure. We treated the movement rates in the surrounding habitat as an expected crossing frequency as this is the movement rate we would expect if no road barrier existed. Therefore, a positive differential indicated that animals moved through the structure more than expected, whereas negative differentials indicated that animals avoided the crossing structures.

While useful for determining effectiveness, differentials are less useful for biological interpretation as the difference of one individual may be more or less important given the size of the population or rarity of the species. Therefore, we also calculated a percentage difference between rates at the structure and in the surrounding by dividing the differential by the expected rate from the surroundings then multiplying by 100. If the ratio was the undefined value zero over zero, we defined this as a percent difference of zero, since this case represents the same crossing rate at the structure as expected from the surrounding. The percent difference centered at zero, and positive values indicated positive performance while negative values indicated structure avoidance.

We estimated average performance measures (differential and percentage difference) for each structure from the data and used nonparametric bootstrap resampling procedures over 100,000 iterations to construct bias corrected and accelerated (BCa) 95% confidence intervals at each structure using the *boot* package in R (Canty and Ripley, 2016). Values were calculated for the four most common species (white-tailed deer, mule deer, black bear, and coyote) and groups of animals. Groups included a deer group (*Odocoileus* sp.), a carnivore group (order *Carnivora*), and a large mammal group (all large mammals observed; **Table 2**). Observation rates decreased as the large mammal group was split into subsets and then into species. We excluded subset or species with fewer than two observations per structure over the sampling period.

We calculated an overall measure of performance across all structures to determine the total effectiveness for each species and group by taking the mean performance of each structure weighted by the number of observation days at each site, then used a weighted one-sample *t*-test to test if the overall differential was zero, along with Bonferroni corrections. In order to construct confidence intervals for these estimates, we employed a multistage, nonparametric bootstrapping procedure, first resampling daily performance within structures with 10,000 iterations, then resampling average performance between structures with 10,000 iterations to yield an overall BCa 95% CI for each species and group. Structures that were excluded from the previous analysis by structure were also excluded from the overall analysis.

In order to assess the impact of wildlife exclusion fencing on structure performance rates, we conducted a linear regression analysis on the relationship between fencing length and structure performance, weighted by the number of full 24-h days of observation recorded at the location. For this analysis, we defined the fence length associated with a structure as the road-wise distance to the nearest alternative crossing. Alternative crossing opportunities included the next suitable crossing structure if

TABLE 2 | Details on animal movements observed at underpass sites and number of full, 24-h days recorded at each site.

Site name	Site code	Days (n)	Obs. Location	Large mammal	Deer	Carnivore	*O. virginianus*	*O. hemionus*	*U. americanus*	*C. latrans*
				Movements recorded *(n)*						
North Evaro	NEV	14	Struct.	16	16	0	16	0	0	0
			Surr.	39	38	0	38	0	0	0
			Total	55	54	0	54	0	0	0
Finley Creek #1	FC1	14	Struct.	179	177	2	177	0	2	0
			Surr.	100	94	4	94	0	1	2
			Total	279	271	6	271	0	3	2
Finely Creek #2	FC2	20	Struct.	36	31	5	31	0	5	0
			Surr.	274	249	21	249	0	17	3
			Total	310	280	26	280	0	22	3
Finely Creek #3	FC3	13	Struct.	23	21	2	21	0	2	0
			Surr.	40	36	3	36	0	3	0
			Total	63	57	5	57	0	5	0
Ravalli Curves #1	RC1	20	Struct.	145	127	18	59	68	0	17
			Surr.	171	161	10	41	120	1	9
			Total	316	288	28	100	188	1	26
Ravalli Curves #2	RC2	14	Struct.	217	216	13	0	214	0	0
			Surr.	162	146	1	112	30	7	6
			Total	379	362	14	112	244	7	6
Copper Creek	COPC	12	Struct.	6	0	6	0	0	6	0
			Surr.	28	18	10	13	5	6	2
			Total	34	18	16	13	5	12	2
Ravalli Hills #1	RH1	14	Struct.	44	41	3	0	41	3	0
			Surr.	80	72	7	0	72	1	5
			Total	124	113	10	0	113	4	5
Ravalli Hills #2	RH2	13	Struct.	3	3	0	0	3	0	0
			Surr.	30	23	7	0	23	0	7
			Total	33	26	7	0	26	0	7
Pistol Creek #1	PIC1	13	Struct.	4	2	1	2	0	0	1
			Surr.	43	35	7	35	0	0	7
			Total	47	37	8	37	0	0	8
Pistol Creek #2	PIC2	12	Struct.	4	4	0	4	0	0	0
			Surr.	35	24	11	24	0	0	11
			Total	39	28	11	28	0	0	11
Sabine Creek	SABC	13	Struct.	22	22	0	22	0	0	0
			Surr.	259	252	7	252	0	3	4
			Total	281	274	7	274	0	3	4
Post Creek #1	POC1	12	Struct.	52	52	0	51	0	0	0
			Surr.	196	195	1	195	0	0	1
			Total	248	247	1	246	0	0	1
Post Creek #2	POC2	13	Struct.	58	58	0	58	0	0	0

(Continued)

TABLE 2 | Continued

Site name	Site code	Days (n)	Obs. Location	Large mammal	Deer	Carnivore	*O. virginianus*	*O. hemionus*	*U. americanus*	*C. latrans*
				Movements recorded *(n)*						
			Surr.	248	241	7	241	0	0	7
			Total	306	299	7	299	0	0	7
Post Creek #3	POC3	13	Struct.	106	99	0	99	0	0	0
			Surr.	181	175	4	175	0	0	4
			Total	287	274	4	274	0	0	4

At each site, the location of the observations is indicated as those recorded at the structure (Struct.), those recorded in the surrounding control plots (Surr.), and total observations at the site.

fencing was continuous or the fence end if discontinuous. If no fence was associate with a structure, or if the fence extended only from one side of a structure, we assigned a value of 0 m as the distance to nearest alternative crossing (**Table 1**).

Although structures were specifically selected for their similarity, the total length of the structures varied with the road width (**Table 1**). We tested for a relationship between structure performance and structure length by conducting a linear regression analysis weighted by observation days similar to the above analysis of fencing distances.

Finally, we used the average percent difference in movement rates to estimate the requisite percentage of road length that must be permeable in order to allow similar movement rates through the road corridor as in the immediate surroundings (**Table 3**). This is the total road length that must include structures of the design considered here, in order to maintain connectivity for those animals willing to come close to the highway.

RESULTS

Wildlife Observations

Each structure and associated control plot was monitored for a median of 14 days (range = 12–20 days) between April and November 2015 (**Table 2**). A total of 2,798 unique large mammal movements were recorded. Twenty-five animal species were observed (**Table 4**). The cameras recorded mountain lions (*Puma concolor*) on two occasions, however they fell on non-24 h days, and thus, were not included in the analysis.

Of the total movements, 886 (30.3%) were recorded at structure entrances and 2,040 (69.7%) were recorded in control plots. The number of movements recorded varied among sites (mean = 193, max = 381, min = 34), as did the number of movements per day (mean = 1.22, SD = 0.84).

Structure Performance

Overall, structures exhibited significantly positive performance with all species and species groups moving through the crossing structures more often than expected based on movement rates in the surrounding habitat (**Figure 3**). Of the 15 total structures, 11 exhibited positive performance measures for large mammals, six of which were significant. Performance for large mammals was significantly negative at only 1 structure (SABC). On average, we observed 146% more large mammal movements per day at the structures than would have been expected based on movements in the surrounding habitat (BCa 95% CI [27.7, 254.8]) corresponding to 1.38 more movements per day (BCa 95% CI [0.26, 2.40]) (**Table 3**).

TABLE 3 | Performance measures (average daily differential and percentage difference) for each species and species group and estimated percentage of road permeability required for 100% connectivity through the road corridor compared to movement rates in immediately surrounding habitat.

Species	Sites (n)	Δ	$\Delta\%$	*p*-value (Bonferroni corrected)	Permeability (%)	Permeability (95% BCa CI) (%)
Large mammals	15	1.38	146	<0.001	40.67	28.81, 78.33
Carnivores	14	0.05	89	0.052	52.94	25.85, 100
Deer	15	1.32	150	0.000	40.04	25.69, 70.50
White-tailed deer	12	0.74	88	<0.001	53.12	22.86, 61.46
Mule deer	5	1.86	472	0.001	17.49	7.65, 53.85
Black bear	7	0.05	112	0.351	47.22	35.24, 100
Coyote	13	0.03	75	0.702	57.07	17.54, 100

As groups, performance across all structures was positive for both deer and carnivores with 149.8% (BCa 95% CI [41.8, 289.2], $p < 0.001$) and 88.9% (BCa 95% CI [−38.7, 227.3]) more movements than expected, respectively (**Table 3**). However, the relationship was not significant for the carnivore group after Bonferroni corrections ($p = 0.052$). For deer, performance was positive at 10 structures, and negative at five (**Figure 3**). Carnivores were present at 14 structures, of which 6, a minority, exhibited positive performance (**Figure 3**).

When considered for each species, performance was positive and of similar magnitude for white-tailed deer ($\Delta\% = 88.2$, BCa 95% CI [62.7, 337.4], $p < 0.001$], black bear ($\Delta\% = 111.8$, BCa 95% CI [−49.0, 183.8], $p = 0.35$), and coyote ($\Delta\% = 75.2$, BCa 95% CI [−73.4, 470.2], $p = 0.70$]; however, the differential was not significantly different from zero for either carnivore species (**Table 3**). Performance for mule deer showed the most highly positive difference with 471.7% more movements through the structures than expected (BCa 95% CI [85.7, 1207.8], $p = 0.001$; **Table 3**].

Performance for all species was considerably variable across structures (**Figure 3**). Performance for coyotes was the most consistent with negative performance at 12 of 13 structures.

TABLE 4 | Total observations and group percentages of animal movements at both structure and control plots (combined total) in the study along US93 within the Flathead Indiana Reservation, Montana for April through November 2015.

Species	Number of observations	Percent of wild animal observation (%)	Percent of large mammal observations (%)	Percent of deer observations (%)	Percent of Carnivore observations (%)
White-tailed deer (*Odocoileus virginianus*)	2047	70.0	73.2	77.8	
Mule deer (*Odocoileus hemionus*)	576	19.7	20.6	21.9	
Deer sp. (sp. undetermined)	7	0.2	0.3	0.3	
Moose (*Alces alces*)	2	0.1	0.1		
Elk (*Cervus canadensis*)	1	0.0	0.0		
Black bear (*Ursus americanus*)	57	1.9	2.0		34.5
Bear sp. (sp. undetermined)	1	0.0	0.0		0.6
Coyote (*Canis latrans*)	86	2.9	3.1		52.1
Bobcat (*Lynx rufus*)	6	0.2	0.2		3.6
Raccoon (*Procyon lotor*)	10	0.3	0.4		6.1
Skunk (*Mephitis mephitis*)	4	0.1	0.1		2.4
American badger (*Taxidea taxus*)	1	0.0	0.0		0.6
Mountain cotton-tail rabbit (*Sylvilagus nuttallii*)	23	0.8			
Red squirrel (*Tamiasciurus hudsonicus*)	3	0.1			
Bat (sp. undetermined)	2	0.1			
Ring-necked pheasant (*Phasianus colchicus*)	47	1.6			
Turkey (*Meleagris gallopavo*)	13	0.4			
Grouse (sp. undetermined)	1	0.0			
Magpie (*Pica hudsonia*)	10	0.3			
Great Blue Heron (*Ardea herodias*)	1	0.0			
Western meadowlark (*Sturnella neglecta*)	1	0.0			
Starling (*Sturnus vulgaris*)	15	0.5			
Red-tailed hawk (*Buteo jamaicensis*)	1	0.0			
Canada goose (*Branta canadensis*)	2	0.1			
Great-horned owl	1	0.0			

(Continued)

TABLE 4 | Continued

Species	Number of observations	Percent of wild animal observation (%)	Percent of large mammal observations (%)	Percent of deer observations (%)	Percent of Carnivore observations (%)
(*Strix nebulosa*)					
Bird sp. (sp. undetermined)	8	0.3			
Data collector	63				
Human	54				
Cow	1783				
Dog	31				
Cat	31				
Total Obs. (Percent of wild animal observations)	4888	2926 (100%)	2798 (95.6%)	2630 (89.9%)	165 (5.6%)

Performance was generally positive for white-tailed deer (seven of 13 structures), black bear (five of seven structures), and mule deer (three of five structures). Performance for mule deer showed the greatest range with 3110% more crossing than expected at RC2 and 100% fewer crossings at COPC. Only one structure, RC1 showed consistently more crossings than expected for all species. Three structures, RH2, PIC1, and SABC showed consistently less crossings than expected. At most structures, performance was split by species.

The distance to the nearest crossing alternative, due to exclusion fencing, was the one variable that we would expect to affect all species similarly as it is a physical barrier intended to funnel animals to the crossing structures (Huijser et al., 2016b). However, a regression weighted by observations days to test the association between mean performance of large mammals and the distance to the nearest crossing alternative was insignificant ($\beta = 0.08$, $R^2 = 0.004$, $p = 0.17$; **Figure 4**). In fact, removing one site with high leverage (RC1) produces a significantly negative, although very weak, slope ($\beta = -0.35$, $R^2 = 0.05$, $p = 0.001$). Similarly, the association between structure length and mean performance of large mammals was insignificant ($\beta = 1.42$, $R^2 = 0.002$, $p = 0.50$; **Figure 5**).

Permeability of Road Corridor

We estimate that in order to allow full connectivity for large mammals, the road corridor must include crossing structures of the design considered in this study along 40.7% of the total road length (**Table 3**). To illustrate this point, consider an example wherein large mammals are observed in the surroundings at a rate of 2.460 per unit time vs. 1 per unit time at the structure. This is a percent difference of 146% (corresponding to the observed Δ% in performance for large mammals found in this study). Since both the rate at the structure and in the surroundings were calculated for the same observational distance (10 m), each unit of crossing width permits the same connectivity as 2.460 units of distance in the surrounding; or, inversely, every 0.407 unit of crossing structure width allows the same movement rate as 1 unit distance in the surrounding. Thus, if the goals is to allow enough connectivity to maintain the same movement rates through the structure as in the immediate surroundings, 40.7% of the total road length must be permeable.

We estimate that including structures of this design along 28.2 to 78.6% of the total road corridor would allow full connectivity for all four of the focal species considered in this study (**Table 3**).

DISCUSSION

Wildlife crossings can provide conservation value in many ways and at many scales; however, determining the success of a given project depends on the intended purpose of the crossing structure(s) (Clevenger and Waltho, 2005). In the case of the US 93 highway upgrade project, mitigation efforts had two goals: minimize wildlife-vehicle collisions and maintain or improve habitat connectivity for wildlife by providing safe crossing opportunities (Marshik et al., 2001; Hardy et al., 2007; Huijser et al., 2016a). For this study, we defined success as performance differentials greater than or equal to zero, meaning that animals were at least as likely to move through a crossing structure as adjacent habitat and did not avoid traveling through the structures.

Overall, for large mammals as a community and all individual species, structures exhibited positive performance, or at least, did not exhibit significant evidence that performance was negative. This suggests that the structures were successful in one of their intended purposes: to maintain or improve habitat connectivity for wildlife by promoting movement across the road corridor through crossing structures. It should be noted that this project-wide analysis applies only to sampled road lengths that necessarily include underpasses. This is only a fraction of the total road length in the study area. In other words, the project may be successful in reducing fragmentation where crossing structures have been installed, but not necessarily over the entire road length. For example, if no animals cross the road at-grade, one would still need to make 40.7% of the road length permeable to eliminate the barrier effect for large mammals willing to come close to the road. In the context of the 90 km US 93 project, that would translate to an installation density of 3.7 10 m wide

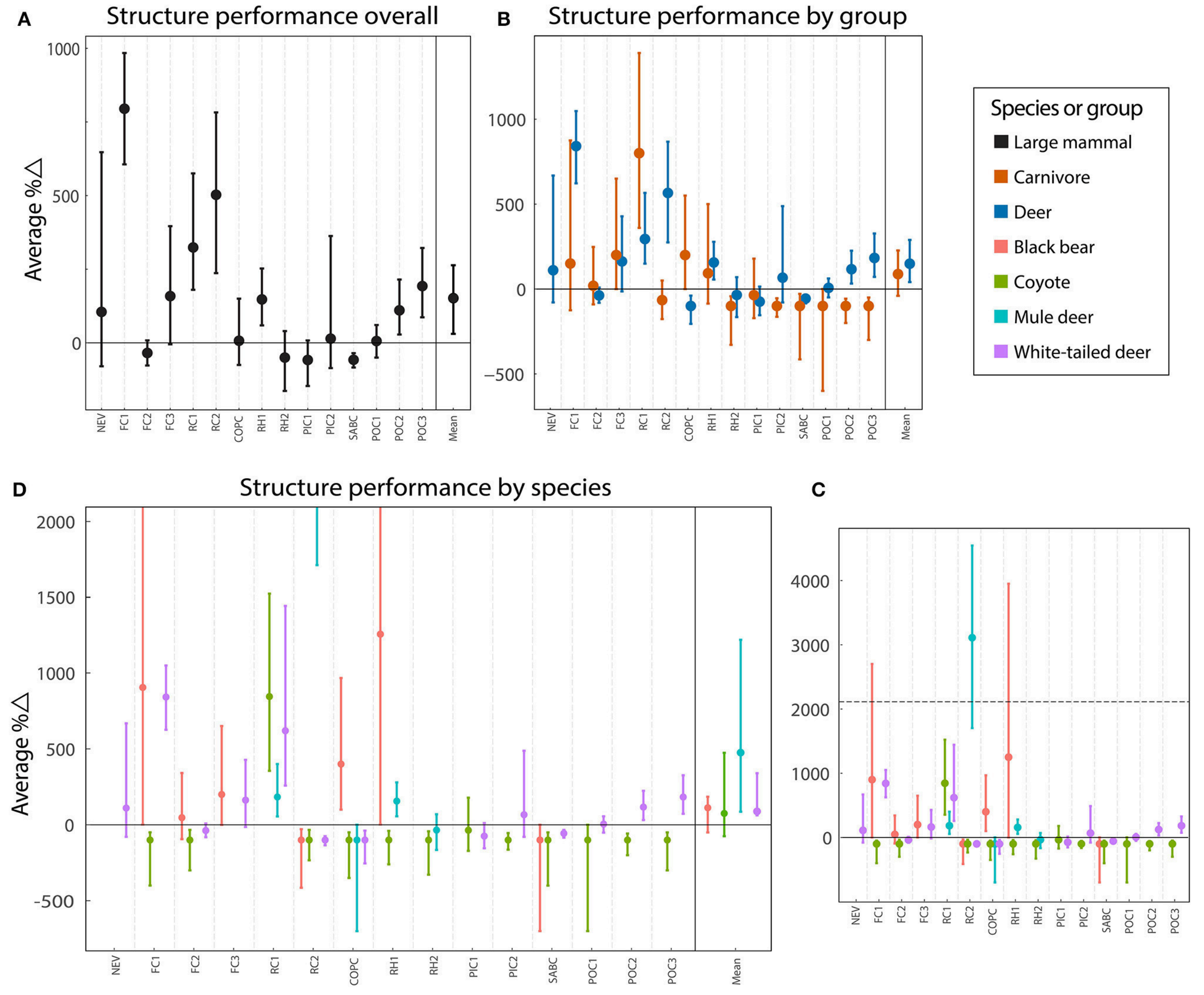

FIGURE 3 | Average percent difference in performance between movements at wildlife crossing structures and in the surrounding habitat for each site and all averaged across sites for species and species groups. Observations are subdivided from all large mammals **(A)**, to species groups (carnivore and deer) **(B)**, to individual species (black bear, coyote, white-tailed deer, and mule deer) **(C)**. To make smaller values easier to see, the y-axis in the plot displaying performance by species has been expanded and truncated **(D)**. The horizontal, dotted line in **(C)** indicates the truncation point for **(D)**. Weighted mean values across all applicable sites are included in the far right of the panels. Error bars indicate bootstrap BCa 95% confidence intervals.

structures per kilometer of road. Currently, the total width of all crossing structures on this section of US 93 is 573 m (Huijser, *unpublished data*), resulting in just 0.6% permeability. Granted, other structure designs included in the US 93 project may permit more or less large mammal connectivity. Performance data are needed for the other structure designs in order to calculate true percentage permeability permitted along the entire road corridor.

Many studies have shown the importance of crossing structure size/design on passage rates (e.g., Yanes et al., 1995; Clevenger and Waltho, 2000; Ascensão and Mira, 2007) while others have demonstrated that location is most important (Foster and Humphrey, 1995; Land and Lotz, 1996). To help illuminate this question, we investigated the effects of location when such structural design parameters are fixed. Although performance was positive for all scales analyzed from community to individual species, the performance of individual structures for species passage was highly variable. This demonstrates that even congruent structures of the same design can yield very different performance, suggesting that location may be more important than design.

Very few wildlife-focused road projects include multiple structures of similar designs to adequately compare as replicates (van der Ree et al., 2007). Given the variability demonstrated by our structure type, this highlights the need for caution in extending conclusions about performance of structure or

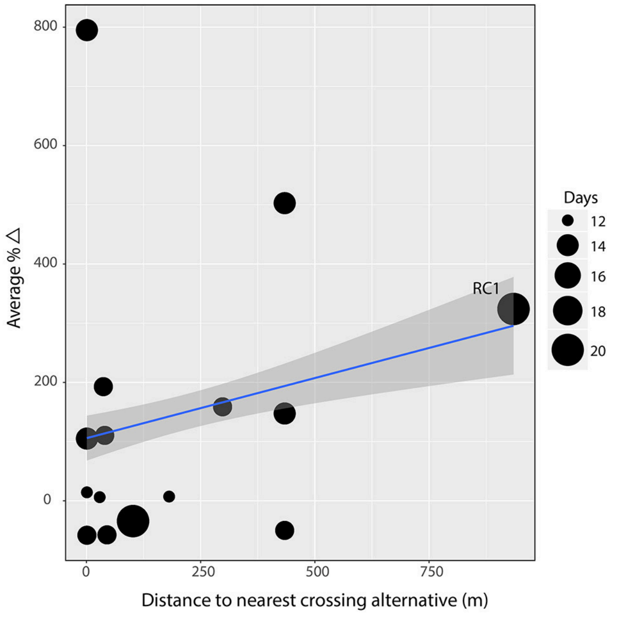

FIGURE 4 | Linear regression on weighted average percent difference in performance against the distance an animal must travel away from the crossing structure at the site to access the nearest alternative crossing opportunity. There is no evidence ($\beta = 0.08$, $R^2 = 0.004$, $p = 0.17$) that the distance (in meters) an animal must travel to find an alternative crossing associates with performance. Gray band indicates 95% confidence interval and point size indicates number of days of observation at the site. The data point for site Ravalli Curve #1 (RC1) exhibited high leverage. When removed, the slope becomes significantly negative ($\beta = -0.35$, $R^2 = 0.05$, $p = 0.001$).

location attributes from studies of a single structure or groups of unreplicated structure designs.

A persistent concern in wildlife crossing design is the potential for underpass structures to act as prey traps causing prey species to avoid structures utilized by predators (Little et al., 2002). If prey species had learned to avoid structures with high predator concentration we would have expected to see opposite performance between prey and predators at individual structures. We did not see this trend; in fact, at half of all structures used by both carnivores and deer, the sign of the performance measure was the same for both groups. It is possible that prey species have coordinated underpass use to time periods when predators are absent, a pattern that would not be resolved at the daily timescale recorded in this study. However, Ford and Clevenger (2010) specifically tested for this type of behavioral assortment and found no such temporal correlation of use between predator followed by prey and the converse for either <8 or <48 h intervals. Interestingly, some of the highest performance values for both black bear and white-tailed deer were observed at FC1, granted black bears are not the most significant predator for adult deer. Multiple alternative crossing, including one vegetated overpass and three elliptical, arch-style wildlife underpasses, are located within just a few hundred meters of FC1. Despite ready alternative crossings, these species did not appear to selectively assort into different crossing preferences.

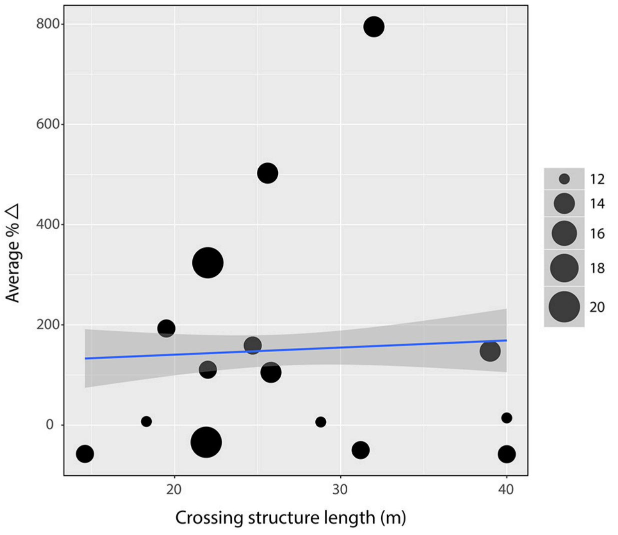

FIGURE 5 | Linear regression on weighted average percent difference in performance against structure length. There is no evidence ($\beta = 1.42$, $R^2 = 0.002$, $p = 0.50$) that the length (in meters) of a structure associates with performance. Gray band indicates 95% confidence interval and point size indicates number of days of observation at the site.

Similarly, FC1 was the only structure through which livestock regularly passed, whereas the four proximal alternative crossings exclude livestock but permit deer and bear. Although more data is needed, this suggests that at certain sites, the coincident use of a structure by livestock may not be a significant deterrent to some species of large mammals as is often assumed (Ruediger and Jacobson, 2013).

When performance for large mammals overall is considered in association with only fence length (represented as distance to nearest alternative crossing), there is no evidence of an effect. If wildlife fencing is truly funneling wildlife to the structures, we would have expected to find higher crossing numbers through the structures with increasing fence length. Huijser et al. (2016b) found that wildlife fencing along US93 shorter than about 5,000 m was less effective than longer fencing at reducing wildlife vehicle collisions. The maximum fenced length to the nearest crossing alternative in our study was under 1,000 m. It should be noted that, Huijser et al. (2016b) considered total fencing length, whereas our study only considered distance to the nearest alternative crossing point. It may be that animals were selecting alternative structure designs instead of the structures in this study, or that animals have become habituated to cross the road corridor less in sections where fencing is a barrier. It is also worth noting that Huijser et al. (2016b) included only isolated structures in their analysis whereas this study includes both isolated structures and those connected by fencing to other structures, many of different design than considered here. Until a similar performance analysis has been conducted on the other structure designs connected to the focal structures, it is impossible to determine the absolute effect of fencing length on performance. For instance, site RC1 in this study has the greatest distance to an alternative crossing (935 m), almost double that of any other site. RC1 also exhibited the highest leverage in the regression analysis, and when removed, led to an opposite result. Analyzing more structure with a range of fencing lengths, or experimentally manipulating fencing length may help to isolate the effect of fencing length for structure performance.

Generally, the results from this association study should be interpreted in context. No observational study can assign causation, and correlation may be confounded because structures are not placed at random in a road. Planners use wildlife vehicle collision, carcass counts, biological surveys, and biological inference to determine the best placement of structures (Huijser et al., 2008). Political and physical limitations further refine where a structure is ultimately placed. As such, the intentional placement of structures invariably correlates structures with locational attributes.

While the use of camera traps as a method of recording animal movements afforded an understanding of general trends within the focal large mammal populations, it did not allow for tracking of individual animal behavior. As such, we were not able to conclusively determine if a particular specie's behavior, such as, a proclivity to forage in a single location, influenced our estimates. Although, we believe that the 5 min threshold between observations of conspecifics mitigated most of this potential error.

The strength of this study comes from controlling for relative abundance, spatial and temporal variability, and variability of structure design. Furthermore, replication allowed for strong statistical inference. Using identical cameras to record both observed and expected crossing frequencies negated any detection variability. Sampling immediately adjacent to the road controlled for habitat variability between the crossing structure and surroundings. This pilot study suffered from limited sampling periods at each site as a trade-off in favor of rapid assessment of many structures. This disallowed strong conclusions about more cryptic taxa like black bear, bobcat, elk, moose, and mountain lion. Longer sampling periods with replicate sampling of the same structure in different seasons would strengthen a future study.

In the absence of large-scale, manipulative experiments to test structure design and locational attributes in controlled settings, the field of road ecology must utilize rigorous observational, control-impact design. More replicates of structure variables will translate to greater power to detect associations with performance. With every new wildlife-friendly road construction project, new potential data points enter the field. In order to ensure that these new data points can be utilized as replicates in statistical analysis of the effectiveness of structure design, we suggested that subsequent crossing structure assessments utilize sampling methodology similar to this study that controls for temporal and spatial variability and variability in detection rates.

AUTHOR CONTRIBUTIONS

AA designed, collected data, analyzed, and wrote up this study. MH designed and wrote-up this study. LB designed this study.

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Vegetated Fauna Overpass Disguises Road Presence and Facilitates Permeability for Forest Microbats in Brisbane, Australia

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The effects of urbanization on flying vertebrates historically have been underestimated, with roads in particular impacting bat populations through vehicle collisions, habitat fragmentation and unwillingness to disperse across roads. Although vegetated fauna overpasses have not traditionally been considered as beneficial for microbats, these structures have recently been identified as a potential solution for assisting microbat communities. In Brisbane, Queensland, Australia, the Compton Road fauna overpass traverses a four–lane road, facilitating connectivity for mammals, birds, reptiles and amphibians between two forest reserves. This study examined patterns of activity and species richness of microbat assemblages in relation to the Compton Road fauna overpass. Call recordings made over 7 months identified nine species and two species groups (groups of species unable to be differentiated), all of which were recorded using the overpass. Bat activity on the overpass was higher than in the adjoining forest, with higher detectability. Species richness and activity at the roadside where the overpass was present was also higher than the unvegetated roadside. Activity patterns based on feeding behaviors suggest that forest species such as *Nyctophilus* and *Myotis* spp. were using the overpass, alongside more generalist species. The vegetation on the overpass is likely to be the key factor encouraging microbat activity. The Compton Road overpass appears to lessen the impact of road presence and may facilitate permeability of a major road, suggesting potential for enhancing habitat connectivity and facilitating crossings. This study is the first indication that vegetated fauna overpasses can facilitate activity and provide natural habitat continuity for diverse Australian microbat communities.

Keywords: fauna passage, overpass, land bridge, *Yangochiroptera*, road ecology, habitat connectivity

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INTRODUCTION

The effects of urbanization on highly mobile flying vertebrates, such as bats, have been historically underestimated (Threlfall et al., 2013), in part from the assumption that they are less influenced by habitat fragmentation than are non-volant species (Gilbert, 1989). Realization of this misconception has facilitated increased research efforts on volant species (e.g., birds: Pell and Jones, 2015; bats: Lesinski, 2008; Bennett and Zurcher, 2013), ultimately showing that urbanization, and the subsequent decrease in habitat connectivity, reduction of remnant habitat and increased anthropogenic disturbance, can reduce species richness, abundance and activity in volant species including bats (see review by Russo and Ancillotto, 2014). Roads in particular, have been shown to directly influence bat mortality (Medinas et al., 2013) by posing significant barriers to movement (Abbott et al., 2012; Bennett and Zurcher, 2013) and restricting dispersal for roosting and foraging (Abbott et al., 2012). Bat density and diversity have also been shown to decrease with proximity to major roads (Berthinussen and Altringham, 2012) while the presence of vehicles themselves can restrict foraging and commuting behaviors (Kerth and Melber, 2009; Zurcher et al., 2010; Bennett et al., 2013). Direct effects such as vehicle strikes (Gaisler et al., 2009; Lesinski et al., 2011), street lighting and traffic noise (Longcore and Rich, 2004; Stone et al., 2009; Bennett and Zurcher, 2013) are also known to impact urban bat communities by altering foraging, commuting, roosting and activity times (Stone et al., 2015). For example, increased artificial light is known to dramatically reduce foraging activity and delay commuting behavior in lesser horseshoe bats (*Rhinolophus hipposideros*) (Stone et al., 2009), while road noise greatly impairs the passive listening abilities of greater mouse–eared bats (*Myotis myotis*) (Schaub et al., 2008). Vegetated fauna overpasses have the potential to mitigate these impacts for bats by reintroducing structural habitat complexity, providing safe passage across roads, as has been shown for other taxa (Bond and Jones, 2008; Mata et al., 2008; Jones and Pickvance, 2013; McGregor et al., 2015).

Fauna overpasses are gaining recognition as a key mitigation strategy for increasing or restoring landscape connectivity and permeability affected by roads (van der Ree et al., 2015). Overpasses have traditionally targeted medium to large mammals (Forman et al., 2003; Glista et al., 2009; Clevenger and Ford, 2010); however, the presence of vegetation has been found to enhance utility by non-target taxa such as birds, reptiles and bats, by establishing a continuation of the natural landscape (Bond and Jones, 2008; Pell and Jones, 2015). Fully vegetated overpasses, particularly those which closely resemble the surrounding natural habitat, have been shown to encourage diverse overpass use by non-target species (McGregor et al., 2015) including small mammals (Bond and Jones, 2008), herpetofauna (Bond and Jones, 2008; Mata et al., 2008), birds (Pell and Jones, 2015) and potentially bats (Bach and Muller-Steiss, 2005; Abbott et al., 2015). Although some studies have observed the use of underpasses by bats (Abson and Lawrence, 2003; Bach et al., 2004; Abbott et al., 2012; Berthinussen and Altringham, 2012), overpasses have only recently been suggested as a mitigation tool for potentially improving road permeability for bats (Bach and Muller-Steiss, 2005; Berthinussen and Altringham, 2012; Abbott et al., 2015). The current understanding of overpass use by bats is extremely limited (Altringham and Kerth, 2015), with only one study, to our knowledge, specifically addressing vegetated overpass use by bats (Bach and Muller-Steiss, 2005). A relatively small number of studies have documented bat interactions with over–road structures (e.g., foot bridges) or unvegetated overpasses (Abbott et al., 2012; Berthinussen and Altringham, 2012; Bhardwaj et al., 2017). These studies noted that features such as strategic location, size, connectivity of tree lines and mature vegetation encouraged use of overpasses by bats, whereas road flyovers (high level road bridges) or footbridges, did not facilitate effective road crossings for bats (Abbott et al., 2015; Altringham and Kerth, 2015).

Roadside vegetation directly affects bat behavior (Zurcher et al., 2010). For example, vegetation along road verges may facilitate commuting, while gaps in vegetation such as tree canopies can prevent the establishment of bat commuting routes (Bennett and Zurcher, 2013). Vegetation near roads can also provide protection as well as foraging habitat (Verboom and Huitema, 1997) and increased insect prey (Avila-Flores and Fenton, 2005). Considering the importance of vegetation to the movement (Russo and Ancillotto, 2014) and behavior (Bennett and Zurcher, 2013) of many bat species, it is likely that a key determinant of overpass use by bats is the presence and structure of appropriate vegetation. The Compton Road fauna overpass is a component of a diverse array of fauna crossing structures in south Brisbane, Queensland, Australia, which were constructed specifically to facilitate the movement of medium to large mammals. The overpass has since been shown to provide road permeability for a range of target and non-target taxa including mammals (Bond and Jones, 2008; Taylor and Goldingay, 2012), herpetofauna (McGregor et al., 2015) and birds (Pell and Jones, 2015). The success of this overpass for non-target species in particular, has been attributed to the established vegetation that integrates the overpass into the surrounding forest (McGregor et al., 2015; Pell and Jones, 2015), facilitating habitat continuity across Compton Road. The aim of the present study was to explore whether the Compton Road overpass, as a fully vegetated fauna crossing, facilitates road permeability for microbat species. It was expected that the road would influence bat activity and species richness, the impacts of which may be lessened where the overpass is present.

STUDY SITE

Compton Road is a major urban arterial consisting of two dual lanes, located in southern Brisbane, Queensland, Australia. The speed limit of Compton Road is 70 km/h and current traffic volume is estimated at approximately 10,000 cars daily (Brisbane City Council, 2014). The road bisects Karawatha Forest Reserve (940 ha) to the south and Kuraby Bushland (140 ha) to the north, two of Brisbane's most significant urban bushland remnants. Both reserves provide natural bushland habitat for a range of native flora and fauna, including numerous significant or threatened species (Mack, 2005; Veage and Jones, 2007). A creek line runs throughout the study area, from Kuraby, underneath Compton Road and parallel with the road within Karawatha forest. Some areas of the forest have been cleared for service roads, fire brakes and power lines (see **Figure 2**).

During 2004–2005, the widening of Compton Road from two to four lanes threatened to permanently isolate the remnant forests, as well as increase collisions between wildlife and vehicles (Veage and Jones, 2007). To mitigate these impacts, the road upgrade incorporated the Compton Road fauna array (27°36′53.11′′S, 153°05′03.12′′E) which included a vegetated overpass (**Figure 1**) and two underpasses. The overpass is hourglass shaped, 70 m long, 15 m wide at the midpoint and 20 m wide at the ends. Roadside exclusion fencing (2.5 m high) extends the full length of the overpass and along the length of each forest at the roadside (Bond and Jones, 2008; Jones, 2010). The natural vegetation of Karawatha and Kuraby is classified as dry eucalypt woodland with native heath understories (Veage and Jones, 2007; Jones, 2010). The vegetation structure and composition on the overpass is similar to the surrounding forests and was planted at a rate of 70 shrubs and six trees per 100 m^2 shortly after construction was complete (2005). This planting regime, primarily consisting of mature saplings, was maintained to facilitate the movement of macropods (Jones, 2010). Surveys undertaken in 2009 confirmed that 95% of the planted trees and shrubs had survived (Jones et al., 2011), maintaining compositional similarity with the surrounding forest.

MATERIALS AND METHODS

Bat Detection and Capture

Bat presence and activity were recorded over two consecutive nights each month for 7 months between December 2014 and July 2015 (February was excluded due to adverse weather). Two EchoMeter Touch (EMT) devices (30 m diameter maximum range, Wildlife Acoustics) and corresponding iPad 4s (Apple Technologies) were used to monitor eight walking transects and two stationary points, beginning approximately 1 h after sunset (in varying order). Transect layout was mirrored on each side of the road, while stationary points were located on the overpass apex, approximately 30 m apart, all of which were monitored each night (**Figure 2**). In each forest, two "road transects" (RT) (defined as transects beginning at the managed, and therefore unvegetated, roadsides) were located to the west, one to the east of the overpass (see **Figure 2**). A single eastern transect was monitored due to the impassable nature of nearby terrain/creek line. Additionally, two overpass transects (OT) (defined as transects beginning level with the road side, but located on the overpass) began level with the fence line and extended into the forest (see **Figure 2**). Each walking transect extended 75 m into the forest, at least 75 m from neighboring transects, with each one being monitored for 20 min. Stationary points on the apex were also monitored simultaneously for 20 min each. Stationary points were monitored by personnel (holding the EMT) in order to maintain human presence at all sites. On each survey night, a single EMT was used to monitor each transect by a maximum of two personnel, always beginning from the roadside, but in no regimented order.

FIGURE 1 | Compton Road fauna overpass in 2015 (Photo: M. McGregor).

Road transects started from the unvegetated roadside fence line, extending into the forest, while the overpass transects began in the constructed habitat on the overpass (in line with the fence) and continued into the forest. The important distinction between road and overpass transects was the lack of vegetation at the roadside, meaning while the overpass transects remained within vegetated habitat for the length of the transect, the road transects did not. This difference allowed for two important comparisons. First, comparing the overpass and road transects at the roadside allowed an assessment of variation in bat activity at the roadside in both the presence and absence of vegetation at the roadside. Second, monitoring from the roadside into the forest determined whether there was variation in bat activity on the roadside compared with in the forest.

To ensure that each walking transect occupied 20 min, six stops were made along the length of the transect of approximately 3 min each; data recorded between the stops was also included. Monitoring did not occur under the overpass for safety reasons. Ethical clearance was approved by the Griffith University Animal Ethics Committee (ENV 08/14 AEC).

DATA ANALYSIS

Call analysis was completed using Kaleidoscope V3.1 and SongScope V4.1.3 (Wildlife Acoustics). Identification was based on local call libraries (south east Queensland and northern New South Wales; from Pennay et al., 2004; Churchill, 2009). Search phase calls, consisting of a series of regular sound pulses (Pennay et al., 2004), were used in this study for species identification,

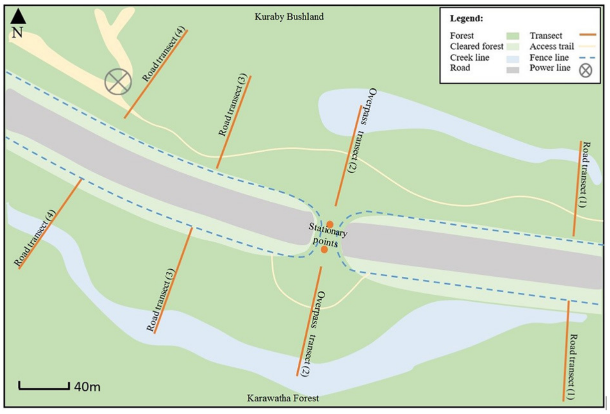

FIGURE 2 | Layout of Eight 75 m walking transects and two stationary points, used to record bat call activity throughout Karawatha Forest and Kuraby Bushland.

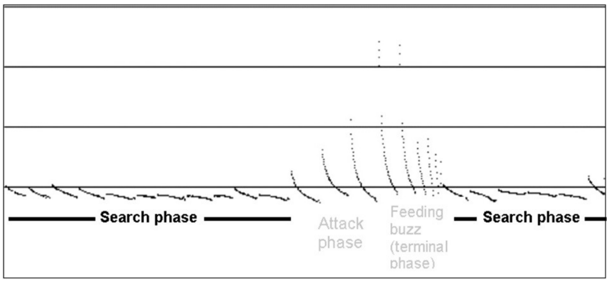

FIGURE 3 | Example diagram shows search phase and feeding buzz call features of a *Mormopterus* species, used for identification during data analysis (figure reproduced from Pennay et al., 2004).

TABLE 1 | Bat species identified from the recorded echolocation calls, including total calls recorded for Karawatha, Kuraby and the overpass.

Taxonomic identification	Karawatha Forest	Kuraby Bushland	Overpass
VESPERTILIONIDAE			
Chalinolobus gouldii[a]	53	85	337
‡Group 1 [*Chalinolobus nigrogriseus*, *Scotoropens greyii* or *Scotoropens species* (Parnaby)][a]	145	150	84
Miniopterus australis[a]	45	13	103
Miniopterus orianae oceanensis[a]	4	33	113
†Group 2 (*Nyctophilus* sp. or *Myotis macropus*)[c]	4	17	18
Scotoropens orion[a]	2	1	4
Vespadelus pumilus[a]	13	6	9
MOLOSSIDAE			
Austronomus australis[b]	10	13	7
Mormopterus beccarii[b]	25	26	21
Mormopterus ridei[b]	65	50	28
EMBALLONURIDAE			
Saccolaimus flaviventris[b]	13	8	2
Species richness total	11	11	11
Activity total	379	402	726

‡*Nyctophilus gouldi, N. bifax, Nyctophilus geoffroyi calls are indistinguishable using bat detectors (Reinhold et al., 2001) and are easily confused with Myotis macropus (pers. comm. B. Thompson).*

†*Similarly, calls from Scotoropens greyii and Scotoropens sp. are indistinguishable from each other (Reinhold et al., 2001; Churchill, 2009), as are the majority of calls from Chalinolobus nigrogriseus lacking the characteristic frequency. Therefore, these calls were grouped.*

[a] *Edge foragers.*

[b] *open space foragers.*

[c] *gleaning foragers.*

while feeding buzzes (a series of often erratic pulses), were used to identify active hunting behavior if occurring within a clearly identifiable call (**Figure 3**).

A complete call was identified by the presence of multiple (three or more), clearly identifiable pulses. Pulse characteristics such as initial sweep shape, body shape and terminal sweep shape (e.g., presence of tail) were used to distinguish between similar species (Pennay et al., 2004). Recordings were identified to species level by M.M. and K.M. independently, while calls that could not be confidently identified were analyzed by a third party for verification. All remaining unidentified calls (less than 5%) were removed from the data set. Calls from species that could not be reliably distinguished from each other, for example *Scotoropens* sp., were grouped together as species groups (Hourigan et al., 2010) (see **Table 1**).

Vocalization analyses focussed on exploring three major trends over the 7 months: microbat activity and richness between the three study areas (Karawatha, Kuraby and the overpass); whether microbat activity and richness varied at the roadside compared with the forest; and finally, whether microbat activity and richness was altered at the roadside by the presence of the overpass. SIMPER analysis (PRIMER 7, PRIMER–E Ltd.) was used to determine the similarity of species activity with distance from the roadside. SIMPER was also used to compare overpass transects to the other transects to determine the impact of vegetation on species similarity at the roadside. Species accumulation curves (SPSS 24, IBM Statistics[1]; Microsoft Excel, 2016[2]) based on richness detected at the beginning and end of the transects were used to explore the likelihood of methodological bias, as well as variation in detectability (likelihood of detecting representative species richness), particularly between the overpass and the forest, and the roadside and the forest. The latter was also used to examine if species richness varied considerably between the roadside and the forest. QGIS 2.10.1 geoprocessing software was used to produce heat maps of total call activity using Google Earth base layers (Google Earth version 7.1.5, 2015) based on Kernel Density Estimation (using night activity as a single data point to determine mean activity over 7 months). Heat map values used call activity numbers and are shown at a radius of 15 m to reflect the maximum potential range of the EMT devices. Transect points were used to display activity within the transect in its entirety and were not treated as discrete data points for analysis, except when compared with each other, to avoid sample bias.

The cryptic nature and high mobility of the study species presented methodological challenges during monitoring. Bat call detection was the only feasible way to monitor sufficient quantities of bats; harp traps were trialed and deemed ineffective due to the lack of captures relative to what was a highly demanding procedure. However, using detectors restricted analysis and application, primarily due to the inability to identify individuals. In this respect, "activity" was explored, rather than abundance, removing any requirement to identify individuals, but was considered a reasonable basis for determining general patterns of bat richness and activity around Compton Road and the overpass. This study is limited to a single study site. Therefore, it relies on replication of transects within the area to determine

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[1] SPSS (V. 22, IBM) IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

[2] Microsoft Excel 2016 (Released in 2016). Microsoft Corporation, Washington DC, United States of America.

patterns of activity and species richness, while replication for monitoring the overpass apex uses two monitoring points for comparison. Due to these limitations, analyses were limited to exploratory and descriptive statistical methods, with a view to helping to construct viable hypotheses that can be tested in future studies.

RESULTS

Nine bat species were reliably identified within the study area from 1,550 clear echolocation call recordings. Clear calls that could not reliably distinguished between species were grouped (Group 1 and Group 2; see **Table 1**). All nine species and both species groups were recorded in Karawatha, Kuraby and on the overpass. Call activity in the two forests were similar, with a total of 387 recorded calls in Karawatha ($\overline{X} = 27.07 \pm 23.34$ SD) and 402 in Kuraby ($\overline{X} = 28.71 \pm 27.46$). Call activity recorded on the overpass (apex points) was almost double (726 calls; $\overline{X} = 51.86 \pm 59.97$) that of the surrounding forest (**Table 1**).

Species Richness and Similarity

Mean overpass calls (stationary points only), averaged throughout the monitoring period, were predominantly comprised of *Chalinolobus gouldii* (24.07 ± 45.28 SD), *Miniopterus orianae oceanensis* (12.56 ± 11.81), and *Miniopterus australis* (8.07 ± 16.55 SD) which were consistently present on every occasion throughout the study. Mean within group species similarity was lowest at the roadside (17.42% similar) and increased in the forest (to a maximum of 57.61% similarity). Dissimilarity was higher between the roadside and the forest ($X = 66.21$) compared with dissimilarity within the forest ($X = 59.376$). Species contribution to dissimilarity between the roadside and the forest was dominated by three species, which were present in all comparisons: Group 1 (mean contribution 30.45%), *Mormopterus beccarii* (mean contribution 25.27%) and *C. gouldii* (mean contribution 13.19%). Calls from *M. beccarii* (34.6%), Group 1 (25.64%) and *C. gouldii* (16.67%) comprised the majority of roadside call activity.

Mean dissimilarity was high between the overpass transects (level with the roadside) and the roadside recordings at all other transects (RT1 = 93.22, RT3 = 77.55, RT4 = 65.85). *C. gouldii* and *Mormopterus ridei* contributed to dissimilarity in all cases (mean contributions 31.06, 24.79% respectively) due to high calls of both species on the overpass transects.

Species Accumulation

Species accumulation quickly reached asymptote within each of the study areas (11 species/species groups) (**Figure 4**). The overpass reached an asymptote sooner than either forest, reaching 11 recorded species on the fourth site visit (January 2015), while Karawatha and Kuraby reached asymptote on the seventh (March 2015) and 10th visits, respectively; however, all three sites displayed similar detection rates.

In comparing species accumulation at the roadside end of each transect to the inner forest end, there was little difference in species richness and species detectability, with the exception of the T2 transect at the roadside (which began on the overpass). The "road" end of T2 transects, in both Karawatha and Kuraby, displayed higher species richness at a faster detection rate than any other forest or roadside point (**Figure 5**).

Call Activity

Call activity was divided into three feeding behavior categories: open foragers (*Austronomus australis*, *M. beccarii*, *M. ridei*, *Saccolaimus flaviventris*); edge foragers (*C. gouldii*, Group 1, *M. australis*, *M. orianae oceanensis*, *S. orion*, *Vespadelus pumilus*); and gleaning foragers (Group 2) (**Table 1**), to determine whether a visual trend could be identified, based on species behavior. All three behavior categories were strongly concentrated on the overpass. Open foragers showed higher activity at the roadside, the creek lines, as well as on the overpass (**Figure 6**). Edge foragers appeared to be spread evenly throughout most transects, while also showing high concentrations on the overpass (**Figure 6**). Gleaning species were rarely recorded within the transects but appeared to be concentrated on the Karawatha side of the overpass and on one Kuraby transect (**Figure 6**).

Calls pertaining to specific behaviors varied slightly between the three areas, with the highest percentage of feeding buzzes (indicating active hunting) being found on the overpass at 4.57%, compared with Karawatha (3.31%) and Kuraby (2.95%). Search phases (indicating no active hunting or interactions between individuals) were the least common call recorded on the overpass, with 11.69%, compared with Karawatha (40%) and Kuraby (20.64%).

DISCUSSION

South east Queensland supports high microbat diversity, with at least 24 known species (Churchill, 1998), 18 of which occur around Brisbane (Churchill, 2009; Hourigan et al., 2010). Previous studies conducted in Karawatha Forest detected at least nine species (Hourigan, 2011), all of which were detected in the present study (although total species richness may be as high as 14, considering indistinguishable call groupings). The presence of microbats on the overpass was substantial, with a high level of activity compared with either forest, and a species richness equal to that found in Karawatha and Kuraby (**Table 1**). Comparisons of species accumulation between the three areas showed detected species richness on the overpass reaching asymptote before either of the forest areas (**Figure 4**). However, both forests did achieve asymptote, suggesting the use of apex point counts on the overpass did not contribute to sample bias, irrespective of using different observation methods. In comparing the overpass transects to the forest transects directly, where the overpass intersects the road had comparatively higher species richness, and high detectability (**Figures 5A,B**), compared to both the unvegetated roadsides and the inner forest. Although the "road" end of the overpass transects had higher species richness and greater detectability than any other roadside or forest records, neither side reached asymptote, suggesting that more species may be using the sides of the overpass, but have not yet been detected. High species detectability, equal species richness and high activity observed throughout the study suggest that diverse microbat communities were using the Compton Road overpass.

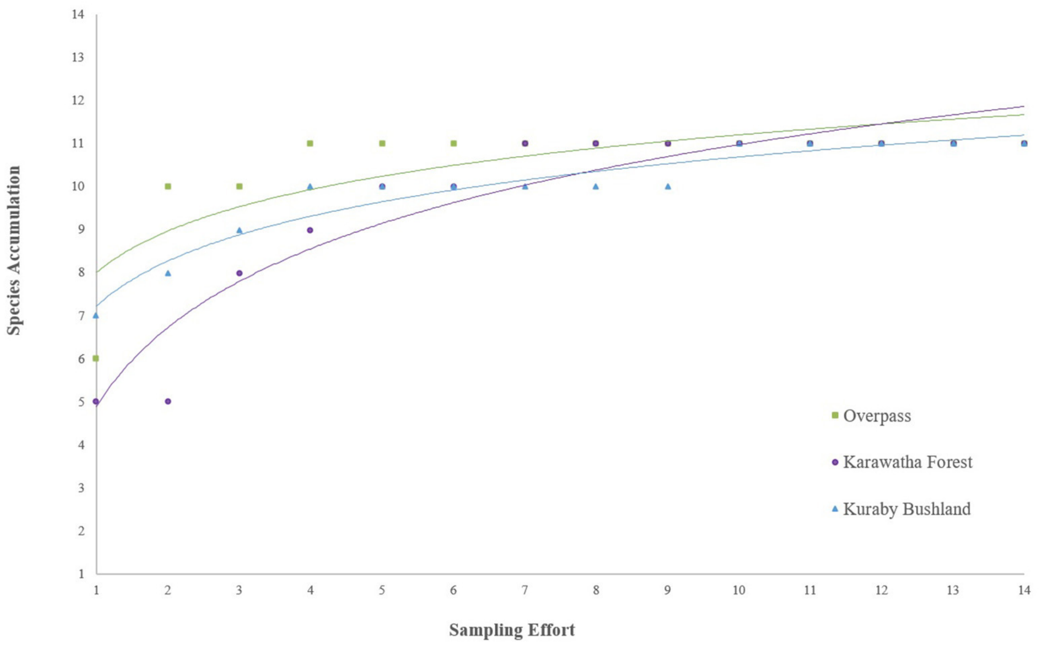

FIGURE 4 | Species accumulation graph comparing detectability and species richness within Karawatha Forest, Kuraby Bushland and on the overpass.

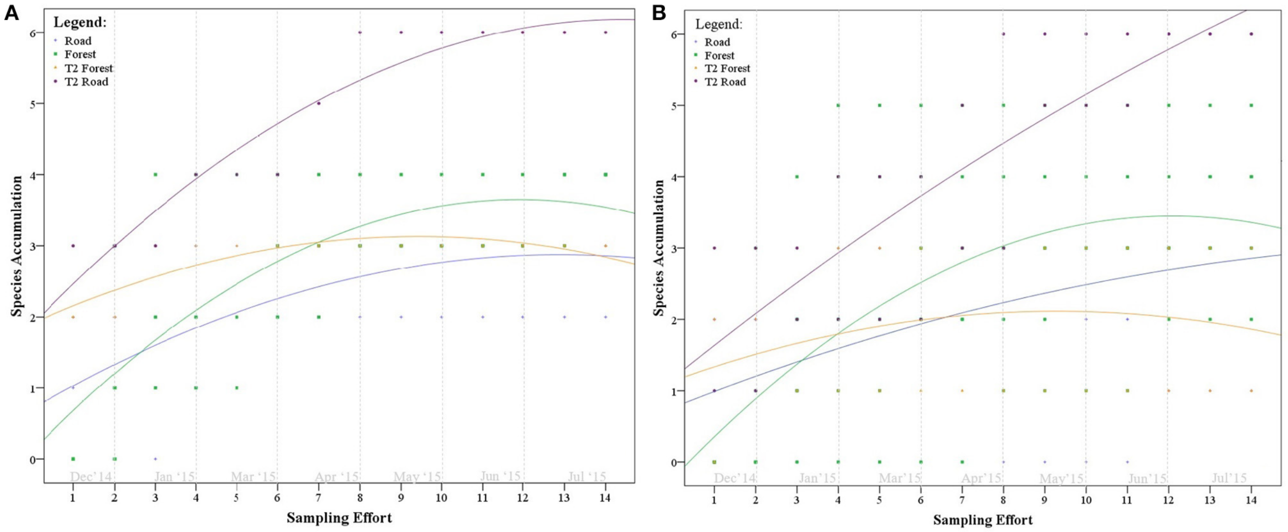

FIGURE 5 | Species accumulation graph comparing detectability and species richness at the roadside and forest ends of each transect (15 m radius) in **(A)** Karawatha Forest and **(B)** Kuraby Bushland.

Contrary to initial predictions, call activity did not appear to vary substantially with proximity to the roadside. Detectability was similar between the cleared roadside and the forest, as was species richness, while the total activity density map shows activity hotspots that did not appear to be correlated with road presence (**Figure 6a**). Typical foraging behaviors were used to explore patterns of activity throughout the study area, based on differences between microbats that may not be affected by road presence, compared with those that are more likely to be susceptible. These groups form the basis for determining how foraging behaviors influence activity patterns, and although some bats such as *Mormopterus* sp. can alter feeding behaviors as required, these groups represent the primary feeding behavior typically displayed by each species. When the data were explored in this way, patterns of activity between bats with typically varied behaviors appear apparent (**Figure 5A**). Call activity along

FIGURE 6 | Call activity along the transects for **(a)** all species, **(b)** open foraging species only, **(c)** edge foraging species, only and **(d)** gleaning species only (mean total calls). Call activity increases from blue to red with intensity (creek and marshland areas outlined in green). *Overpass calls for total species activity are noted separately.

the roadside varied from activity observed in the forest, with most roadside activity dominated by open foragers (*M. beccarii*, *C. gouldii* and Group 1). Forest specialists such as *Nyctophilus* (Group 2) were not specifically correlated with road presence or dense forest. Instead, two hotspots of Group 2 detections were present: transect 4 in Kuraby, and on the overpass. Landscape variation is likely to have contributed to activity patterns observed during the study, a recognized aspect of bat behavior (Bennett and Zurcher, 2013), while it is possible a nearby roost may have skewed activity results. Call activity among all species appears to be positively associated with creek presence and open areas.

Current literature recognizes that roads act as barriers to bat populations (Kerth and Melber, 2009; Berthinussen and Altringham, 2012; Bennett and Zurcher, 2013) by restricting dispersal (Abbott et al., 2012) or forcing changes in behavior, such as road avoidance (Zurcher et al., 2010; Bennett and Zurcher, 2013), although the degree to which this occurs is debated. Berthinussen and Altringham (2011) observed that roads act as considerable, if not complete, barriers to microbat species, while Abbott et al. (2012) suggested that roads of similar construction to Compton Road are not absolute barriers to bat movement. How severely bat communities are affected by road barriers often depends on their morphology (Rydell, 1992; Kerth and Melber, 2009; Bhardwaj et al., 2017). Therefore, observing activity patterns between bats with different foraging behaviors may assist in determining whether, and how, Compton Road affects bat communities living in the surrounding forest. The high levels of bat activity, richness and detectability on the overpass (apex and sides) compared with the cleared roadside suggests that the overpass does provide a level of relief of road presence; however this same trend is notably present when comparing the sides of the overpass with the inner forest. One explanation for this may be the hourglass shape of the overpass creates a bottleneck, restricting bat activity to a narrow area, and therefore concentrating activity to within range of the EMTs. This particular point would benefit from a more intensive monitoring regime which may include monitoring further into each forest.

Bats are known to alter their behavior in response to the structure and availability of vegetation (Bennett and Zurcher, 2013) with suitable vegetation facilitating higher bat diversity (Abbott et al., 2012, 2015). The presence of comparatively high bat activity on the overpass, by all species and groups detected, suggests that the reason for high detectability, richness and activity on the overpass is the presence of complexity in the form of vegetation. The overpass appears to provide habitat sufficient enough to support, and indeed encourage, diverse bat species. This is evident in the comparative concentration of gleaning species activity on the overpass apex, suggesting appropriately complex and suitable habitat for forest specialists. The vegetation on the overpass may be sufficient to reduce negative aspects of the road which are known to affect bats, for example artificial light (Stone et al., 2009) and traffic noise (Schaub et al., 2008), two elements of the road that are noticeably reduced on the apex, although these were not measured in the present study. In addition, the presence of open and edge foragers suggests a planting regime which does not exclude these species, a known consequence of dense vegetation (Medinas et al., 2013). The planting regime employed on the overpass aimed to replicate the local open woodland forest, which may have encouraged overpass use by species rich microbats. To fully understand the benefit of the overpass to bats, ascertaining crossing data by tagging and tracking individual bats would be of great benefit. This would specifically determine whether individuals are using the overpass as a crossing route.

Limitations of the study area size restricted the size of the transects which, due to the potential for road effects to extend far into forest habitat, may have been influenced by the road effect zone. The limited call activity from gleaning species throughout the forest, such as long–eared *Nyctophilus* which are recognized as sensitive forest specialists (Lumsden et al., 2002; Threlfall et al., 2013), compared with far higher call activity from edge feeding free–tailed and sheath–tailed bats, suggests that the road effect zone may extend into the forest, beyond the length of the 75 m transects. This possible outcome is consistent with taxa-diverse studies which suggest road effect zones can extend from 100 to 1,000 m from the road (Reijnen et al., 1995; Eigenbrod et al., 2009), which includes aspects of disturbance (such as noise and light) known to affect bat activity (Schaub et al., 2008; Stone et al., 2009). The broad spread and high call activity from edge foraging species may also suggest that edge effects extend well into the forest, while the dominant presence of Molossidae species, as open-space foragers (Jung et al., 2014), along the roadside indicates substantial influence of road. These patterns suggest that Compton Road may be affecting more sensitive bat species, and is likely to present a complete barrier if this is the case. The presence of *Nyctophilus*/*Myotis* sp. activity on the overpass may provide evidence of increased habitat permeability, and most likely some level of road effect mitigation for these species. It is unlikely that Compton Road impacts open and edge foraging species to the same intensity, although it does not prevent them from being at risk of barrier effects, traffic injury or other road related impacts (Kerth and Melber, 2009).

A variety of management practices have been suggested to reduce road impacts on bat species and potentially prevent further fragmentation. Bennett and Zurcher (2013) recommend management practices that involve interlinking tree canopies across roads, as well as restoring and enhancing vegetation along roadsides. One goal of habitat restoration is to enhance landscape permeability, which is also a primary goal of overpass construction (Forman et al., 2003). Although vegetated overpasses have not traditionally been built or considered for bat communities, the overlap between what an overpass achieves, and what benefits bat communities, appears to be substantial. Bach et al. (2004) concluded that over– road crossing features are more restrictive to bat species than are under–road (typical underpasses as well as culverts) crossings, while underpasses have been suggested as a potential solution to reducing road impacts on some bat species (Abson and Lawrence, 2003; Kerth and Melber, 2009; Boonman, 2011; Abbott et al., 2012). The ecological value of the underpasses at Compton Road has not been determined; however, call activity and species richness on the overpass suggests that the overpass is a substantial asset to the facilitation of habitat continuity across Compton Road.

Current research has highlighted the necessity of maintaining remnant forests within urbanized environments for the long–term persistence of microbats (Avila-Flores and Fenton, 2005; Oprea et al., 2009; Threlfall et al., 2013). The increasing reduction and isolation of patches of remnant forest in urban areas worldwide continues to jeopardize local biodiversity, particularly for species sensitive to habitat fragmentation. Retention of tree cover and linear vegetation, in particular, are critical to the sustainable protection and long–term persistence of urban forests for use by bat communities (Threlfall et al., 2013). Additionally, the presence of overpasses and similar infrastructure that enhances and reintroduces habitat connectivity needs to be appreciated in areas that are already highly fragmented. The Compton Road fauna overpass has shown to be successful in facilitating road permeability for a wide variety of taxa (Bond and Jones, 2008; McGregor et al., 2015; Pell and Jones, 2015); this may now include microbats, with the potential for maintaining habitat connectivity and enabling crossing between Karawatha and Kuraby. Without determining whether bats are crossing the road without the aid of the overpass, it is difficult to quantify "effectiveness" (as defined in Forman et al., 2003; van der Ree et al., 2007; van der Grift and van der Ree, 2015). However, this study shows that the overpass itself is being frequented by a diverse range of microbat species. This study is the first indication that a vegetated fauna overpass can facilitate activity and provide road permeability for diverse microbats in Australia, and one of very few worldwide. It is, therefore, significant in demonstrating the potentially important role of fauna passages in facilitating the long–term persistence of microbat communities in urban landscapes.

AUTHOR CONTRIBUTIONS

MM devised, managed and conducted field research and wrote the majority of the final manuscript as well as facilitating the editing and submission processes. KM contributed significantly to expertise required in the field and assisted in correctly writing the technical aspects of the manuscript as well as editing. DJ supervised the field research and writing process, contributing substantially to editing, research design and execution of the project.

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Models of Coupled Settlement and Habitat Networks for Biodiversity Conservation: Conceptual Framework, Implementation and Potential Applications

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Worldwide, the expansion of settlement and transport infrastructure is one of the most important proximate as well as ultimate causes of biodiversity loss. As much as every modern human society depends on a network of settlements that is well-connected by transport infrastructure (i.e., settlement network), animal and plant species depend on networks of habitats between which they can move (i.e., habitat networks). However, changes to a settlement network in a region often threaten the integrity of the region's habitat networks. Determining plans and policy to prevent these threats is made difficult by the numerous interactions and feedbacks that exist between and within the settlement and habitat networks. Mathematical models of coupled settlement and habitat networks can help us understand the dynamics of this social-ecological system. Yet, few attempts have been made to develop such mathematical models. In this paper, we promote the development of models of coupled settlement and habitat networks for biodiversity conservation. First, we present a conceptual framework of key variables that are ideally considered when operationalizing the coupling of settlement and habitat networks. In this framework, we first describe important network-internal interactions by differentiating between the structural (i.e., relating to purely physical conditions determining the suitability of a location for living or movement) and functional (i.e., relating to the actual presence, abundance or movement of people or other organisms) properties of either network. We then describe the main one-way influences that a settlement network can exert on the habitat networks and vice versa. Second, we give several recommendations for the mathematical modeling of coupled settlement and habitat networks and present several existing modeling approaches (e.g., habitat network models and land-use transport interaction models) that could be used for this purpose. Lastly, we elaborate on potential applications of models of coupled settlement and habitat networks in the development of complex network theory, in the assessment of system resilience and in conservation, transport and urban planning. The development of coupled settlement and habitat network models is important to gain a better system-level understanding of biodiversity conservation under a rapidly urbanizing and growing human population.

Keywords: social-ecological system, land-use transport interaction, spatial networks, habitat connectivity, land-use planning, transport planning, conservation planning

INTRODUCTION

Due to human activities, the past century has seen highly elevated rates of species extinctions at the global scale (Ceballos et al., 2015) and strong reductions in species richness at the local scale (Newbold et al., 2015). A large proportion of this biodiversity loss is attributed to land-use change (Millennium Ecosystem Assessment, 2005; Newbold et al., 2015). Important land-use related threats to biodiversity are, amongst others, the development of settlements and transport infrastructure (Salafsky et al., 2008), which has led to the destruction, degradation or fragmentation of natural habitats across the world (e.g., Salafsky et al., 2008; Reed et al., 2012; McDonald et al., 2013; Bennie et al., 2016). The severity of the threat of settlements and transport infrastructure to the survival of many plant and animal species will further increase with a strongly growing and urbanizing global population (Güneralp et al., 2013; United Nations, 2015). It is predicted that a total of 6.3 billion people (i.e., two-thirds of the global population) will live in urban areas by 2050 (United Nations, 2015), the area of settlements will nearly triple between 2000 and 2030 (Seto et al., 2012) and the combined length of roads and railways will increase by 60% between 2010 and 2050 (Dulac, 2013). Given the fact that many urban centers occur in areas with a high biodiversity (Luck, 2007; Luck and Smallbone, 2010), it is a major challenge to prevent these ever increasing rates of urbanization from having a devastating effect on the world's biodiversity. It is therefore of vital importance that we increase our understanding of how the long-term development of settlements and transport infrastructure can be conducted in a way that minimizes the impact on biodiversity.

The development of settlements and transport infrastructure are two strongly interrelated processes (Wegener, 2014). Settlements connected by transport infrastructure (e.g., roads and railways) form intricate spatial networks (i.e., settlement networks), in which changes to either the settlements or the transport infrastructure can bring about changes in other parts of the network (Badoe and Miller, 2000; Levinson, 2008; Israel and Cohen-Blankshtain, 2010). In a similar way, animal and plant populations connected via movement, dispersal or migration (henceforth referred to as movement) can also form large spatial networks (i.e., habitat networks). Also in habitat networks, changes to any part of the network can have profound effects on other parts of the network or on the network as a whole (Galpern et al., 2011). Just as much as modern human societies have become socially and economically dependent on well-connected settlement networks (Axhausen, 2007; Lakshmanan, 2011), many plant and animal species are dependent on well-connected habitat networks for their survival (Crooks and Sanjayan, 2006). However, expansion of a settlement network often threatens the integrity of habitat networks for plants and animals. Urban sprawl and associated land-use changes can destroy or degrade habitats as well as reduce the movement of species between habitats (Salafsky et al., 2008; Pickett et al., 2011; Van Strien et al., 2014). Also transportation infrastructure and traffic can be detrimental to both habitat suitability and connectivity (Forman et al., 2003; Seiler, 2003; Salafsky et al., 2008; Holderegger and Di Giulio, 2010). Paradoxically, human society also depends on biodiversity as driver for the functioning of a healthy ecosystems (Hector and Bagchi, 2007; MacDougall et al., 2013) and for the provision of many ecosystem services (e.g., clean water, crops, water regulation; Isbell et al., 2011; Mace et al., 2012). Due to this mutual dependency, coupled settlement and habitat networks should be regarded as a complex social-ecological system (Berkes and Folke, 2000). Yet, they have not been treated as such in science or practice.

Coupled social-ecological systems often "exhibit non-linear dynamics with thresholds, reciprocal feedback loops, time lags, resilience, heterogeneity, and surprises" (Liu et al., 2007, p. 1513). Ignoring such complexities when managing social-ecological systems can result in ineffective or even counterproductive policies or actions (Levin et al., 2013). Models of social-ecological systems can help us reveal such complexities and ultimately find better ways to conserve biodiversity (Luck, 2007; Milner-Gulland, 2012). The dynamics and processes that exist within either settlement or habitat networks are fairly well studied. Studies on the evolution of or processes in settlement networks have been performed in the field of human geography and inspired by concepts like central place theory (Christaller, 1933) or complex systems theory (Prigogine and Stengers, 1984). Likewise, the interest in habitat networks in ecology originated from research areas like island biogeography (MacArthur and Wilson, 1967), population genetics (Wright, 1943) or metapopulation ecology (Levins, 1969). Although there is thus a long history of studies aiming to reveal interactions in either settlement or habitat networks, few studies have regarded these networks as a coupled system.

To parameterize the relationships that exist between settlement and habitat networks, one can draw upon knowledge and approaches from several fields of research. In the fields of transport and urban planning, studies have assessed how to mitigate the negative impacts of transport and urban development on ecology or environment (e.g., Williams, 2005; Beatley, 2011; Ercoskun, 2012; Nuissl and Siedentop, 2012). The ecological effects of settlements, roads and traffic have been extensively studied in the research fields of urban ecology (Gaston, 2010; Forman, 2014) and road ecology (Forman et al., 2003; Van der Ree et al., 2015; Bennett, 2017). However, within the fields of road and urban ecology, focus has mainly been on specific types of interactions at a local scale. In an overview of trends in road ecology, Van der Ree et al.(2011, p. 1) concludes that "the current situation, with numerous small-scale projects being undertaken independently of each other, cannot provide the information required to quantify and mitigate the negative effects of roads and traffic on higher levels." In the book Urban Ecology, McKinney (2010, p. 304) summarizes that "[...] conservation planning must extend far beyond the very local scales at which most urban ecology is carried out. We must begin to focus much more attention on the many impacts of cities on distant areas at all spatial scales [...]." It thus seems crucial that knowledge of these local scale projects is aggregated and integrated in models of coupled settlement and habitat networks in order to study the dynamics in these networks at larger scales and in a more holistic, system-focussed approach (Ramalho and Hobbs, 2012). Yet, very few studies have actually endeavored to model coupled settlement and habitat networks in a dynamic way.

In order to aid scientists in setting up models of coupled settlement and habitat networks, we present a conceptual framework of such models and discuss several ways in which such models can be implemented and applied. It can be a challenging task to define and parameterize coupled networks representing social-ecological systems (Janssen et al., 2006). In our conceptual framework, we therefore present an overview of key variables that were found to be important for the dynamics in coupled settlement and habitat networks and are thus ideally considered when developing a model of this social-ecological system. Furthermore, from our assessment of key variables, we distil four general recommendations for implementing integrated models of coupled settlement and habitat networks. We also present several existing mathematical modeling approaches that can form the basis of a coupled network model. Lastly, we discuss several ways in which such models can contribute to biodiversity conservation. We see potential applications in the development of complex network theory, in the assessment of system resilience and in conservation, transport and urban planning.

NETWORK DEFINITIONS

Many social-ecological systems can be represented as networks, but the determination of the network's structure and components can present a challenge (Janssen et al., 2006). Therefore, in order to focus our discussion, we need to define settlement and habitat networks in more detail. Networks (or graphs) consist of nodes (or vertices) and edges (or links), which, in the case of spatial networks, "are constrained by some geometry and are usually embedded in a two- or three-dimensional space, and this has important effects on their topological properties and consequently on the processes which take place on them" (Barthélemy, 2011, p. 3). An edge in a network indicates a relationship between nodes. Properties can be ascribed to nodes and edges by assigning weights, expressing for instance area, population sizes or movement rates.

As the relationship between biodiversity and human activities is scale-dependent (Pautasso, 2007), we will focus on settlement or habitat networks at a regional or national scale that encompass tens or hundreds of settlements. The interactions between the networks can, however, be at a local scale. In this article, the term network does not only refer to the abstract, mathematical representations of nodes and edges, but we use the term in a more encompassing way to indicate the connectedness and relatedness of settlements or habitats in a region.

Settlement Networks

The nodes and edges in networks of areas where people live can be defined in several ways, mainly depending on the level of spatial aggregation of the networks (e.g., Buhl et al., 2006; De Montis et al., 2007; Xie and Levinson, 2011). Fine-scale networks encompassing one or a few settlements, usually consist of nodes that represent road intersections and edges that represent the road segments connecting them (referred to as street or road networks; e.g., Buhl et al., 2006; Lämmer et al., 2006). At a regional or national scale, networks encompass many settlements and are usually more aggregated: a node represents a whole settlement and the edges between nodes represent main roads connecting settlements (e.g., Mollanejad and Zhang, 2014) or the flows of people between settlements (e.g., De Montis et al., 2007). For such aggregated networks, nodes need to be identified by delineating settlements, which is not an easy task given the fact that settlements usually do not follow administrative boundaries (Antrop, 2000). Settlements can be delineated making use of, for example, census data, remote sensing or population data, but also with fine-scale street network data (Zhou, 2015). It should be noted, however, that also at national scales, fine-scale road networks have been used to model traffic flows (e.g., Meister et al., 2010). Settlement networks may also be referred to as urban networks (Antrop, 2000). However, as the term "urban" could suggest a focus on cities, we here prefer the more comprehensive term settlement, which includes smaller villages or towns. As human mobility along roads (including highways) is by far the dominant mode of transport (Dulac, 2013), we will focus our discussion on roads, but many of our findings can also be applied to other forms of terrestrial transport (e.g., railways).

Habitat Networks

There are several ways in which nodes and edges can be defined in networks of animal or plant habitats (Urban et al., 2009; Galpern et al., 2011; Rayfield et al., 2011). In general, the nodes represent habitat patches for a certain (group of) species. The delineation of habitats in a habitat network is not always straightforward, especially not for species that are habitat generalist or those that inhabit landscapes where transitions between environmental conditions are gradual and not abrupt (Galpern et al., 2011). Habitat suitability modeling can be employed for habitat patch delineation (e.g., Ramirez-Reyes et al., 2016). The edges in habitat networks represent the (potential) movement of organisms between the habitats (Galpern et al., 2011). In contrast to human movement, which mainly takes place along transport infrastructure such as roads or railways, the movement of most animals is less bound to distinct landscape features. Furthermore, animal movement is notoriously difficult to measure (Kool et al., 2013). For these two reasons, the definition of edges and their weights (expressing for example movement rates) can present a challenge in habitat networks. Computer models can help determine potential movement routes through the landscape (e.g., least-cost paths or current flow maps; Adriaensen et al., 2003; McRae, 2006). These routes are often derived from resistance surfaces, which are raster maps of the study area showing the hypothesized cost of movement through each cell (Zeller et al., 2012; Cushman et al., 2014). Alternatively, transects can be used to quantify the landscape between habitat patches (e.g., straight-line or least-cost transects; Scolozzi and Geneletti, 2012; Van Strien et al., 2012, 2014). Empirical data on animal movement is either collected by tracking the movement of individual animals (e.g., mark-recapture studies, radio tracking, GPS sensors) or with genetic methods (Kool et al., 2013). Note that for the vast majority of the world's regions there is one settlement network, but many habitat networks, since species differ in their habitat or movement characteristics (e.g., Concepción et al., 2015).

CONCEPTUAL FRAMEWORK FOR MODELS OF COUPLED SETTLEMENT AND HABITAT NETWORKS

Our conceptual framework summarizes the main network-internal interactions that can take place in either the settlement or habitat networks (sections Interactions Within Settlement Networks and Interactions Within Habitat Networks, respectively) as well as the key one-way influences of settlement networks on habitat networks (section Influences of a Settlement Network on Habitat Networks) and those of habitat networks on settlement networks (section Influences of Habitat Networks on a Settlement Network). A dynamic feedback mechanism between settlement and habitat networks can only exist if such influences exist in both directions (**Figure 1**). These interactions and influences are important to capture the complexity of the dynamics within this social-ecological system.

To aid our description of the network-internal interactions, we differentiate between the structural and functional properties of both the settlement and habitat networks (Newman, 2003; **Figure 1**), which has also been used to differentiate between different types of habitat connectivity (Taylor et al., 2006). The structural properties of the networks are characterized by the purely physical conditions that determine whether an area is inhabitable for humans or other organisms (i.e., structural properties of the nodes) or suitable to move through (i.e., structural properties of the edges). For settlement networks, buildings and roads are examples of structural properties of nodes and edges respectively. The structural properties of a habitat network for an amphibian could be, for instance, water bodies (nodes) that are at a certain distance from one another (edges). These structural properties do not reflect the actual use of the networks by humans or other organisms. In contrast, the functional properties of networks are determined by variables expressing the actual presence or abundance (i.e., functional properties of nodes) or movement (i.e., functional properties of edges) of people or other organisms in the networks. The structural and functional properties of the network interact with each other.

Interactions Within Settlement Networks

Human artifacts, such as buildings and roads, form the basis of the structural properties of settlement networks. In modern times, these artifacts basically form the prerequisites for people to inhabit a certain region. Their quality and quantity will thus determine the functional properties of the network, i.e., the number of people that live in a certain settlement or that travel between settlements (**Figure 1**). Due to the capacity of humans to alter their surrounding for their own benefit, there are strong spatial and temporal interactions between the structural and functional properties in settlement networks (Wegener and Fürst, 1999; Badoe and Miller, 2000). Changes to road or settlement structure can lead to demographic or traffic flows changes, which in turn can trigger settlement or road development, and so forth (Axhausen et al., 2011; Xie and Levinson, 2011; Wegener, 2014). Such an effect was, for instance, reported for the state of Minnesota, USA by Iacono and Levinson (2016, p. 216), who "find evidence of feedbacks between population changes and the growth of local [road] networks." Also for the railway development in the city of London, UK, during the period 1871-2007, Levinson (2008, p. 19) found that there was a positive feedback between the development of the rail network and population growth in certain neighborhoods: "train service led to a suburbanization of countryside and increased population of new developments, which attracted more railways." Several studies have shown that the establishment of new transport infrastructure, be it roads or railways, can spark growth in the settlements along these infrastructures as well as changes in the locations of organizations and businesses throughout the region (e.g., Kreibich, 1978; Chi, 2010; Israel and Cohen-Blankshtain, 2010). Job opportunities play an important role in the relocation of people. For instance, one of the main drivers of the widespread phenomenon of rural abandonment is the decrease of job opportunities in agricultural areas and increasing opportunities in and around larger settlements (Rey Benayas et al., 2007). These are only some of the many examples of the interactions that can take place within settlement networks.

Settlement network expansion is hardly limited by those natural factors that limit population growth and spread in other species (e.g., resource availability, competition with other species, climate, topography and diseases). Therefore, to prevent their resource use from surpassing sustainable levels (Ostrom, 2009), human societies have implemented systems of governance (i.e., "all processes of governing, whether undertaken by a government, market, or network, whether over a family, tribe, formal or informal organization, or territory, and whether through laws, norms, power, or language"; Bevir, 2012, p. 1).

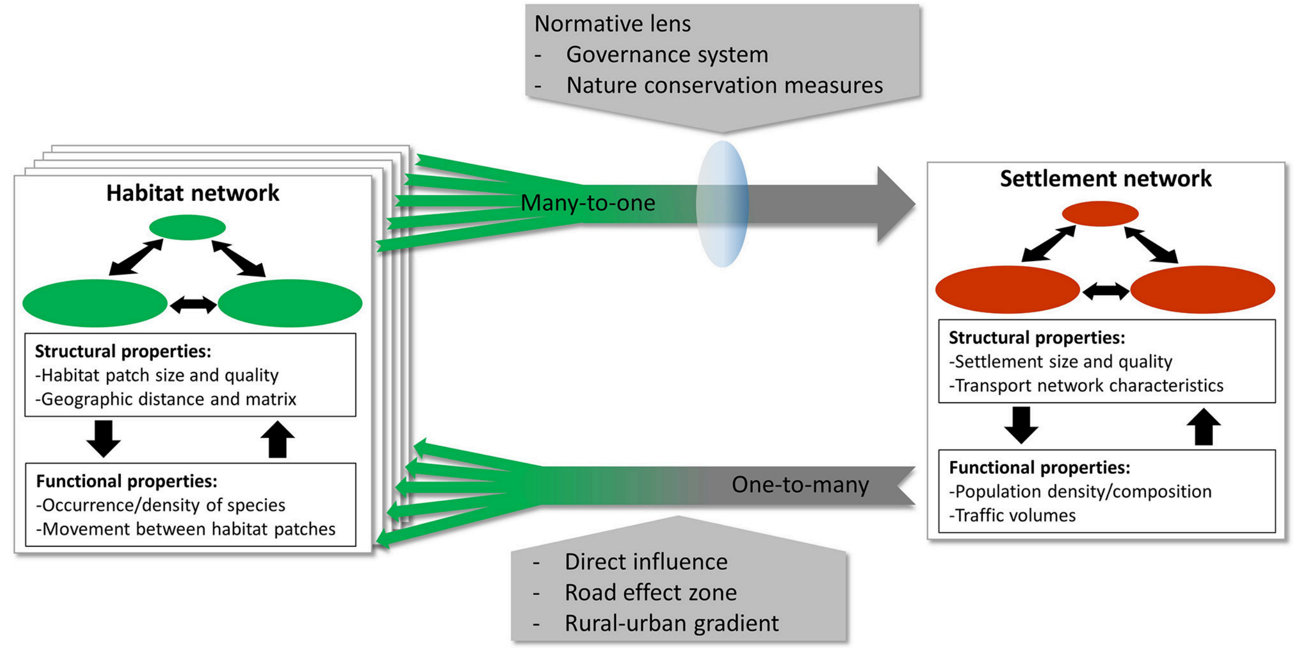

FIGURE 1 | Conceptual framework of models of coupled settlement and habitat networks. The different interactions and influences within and between settlement and habitat networks are shown. Both types of network are embedded in the same landscape. In general there is one settlement network and multiple species-specific habitat networks. Changes in the settlement network often influence several habitat networks (i.e., one-to-many relationship), whereas habitat networks usually only influence the settlement network if change takes place in multiple networks (i.e., many-to-one relationship). Network-internal interactions are between structural and functional properties of the networks. Changes to the structural or functional properties of settlement network can have a direct influence on habitat networks, but also an indirect influence via a range of effects that radiate out from roads (i.e., road-effect zone) or from settlement centers (i.e., urban-rural gradient). The influence of the habitat networks on the settlement network is rarely direct, but usually only arises once nature conservation measures are installed, which are a result of the implemented governance system and influenced by the normative lens through which society views biodiversity and its conservation.

In settlement networks, governance systems can regulate, for instance, traffic or land allocation. Empirical evidence indicates that traffic can be regulated with a range of policy actions, such as land-use planning, pricing of roads or of parking, increasing the attractiveness of public transport or awareness campaigns (Graham-Rowe et al., 2011; Salon et al., 2012). As changes in mobility patterns can have consequences for location choices of households, companies and organization, these policy measures may cause unforeseen effects on patterns of built-up land-use in the future (Goodwin, 1998), but can also be used as an effective measure to steer settlement development (Nuissl and Siedentop, 2012). Other governance systems to regulate settlement development can be based on planning (e.g., zoning, density controls or transport planning), management (e.g., information campaigns or forums) or market forces (e.g., development taxes, subsidies or tradable permit schemes; Nuissl and Siedentop, 2012).

Interactions Within Habitat Networks

In order to describe the interactions within habitat networks, it is important to define the functional and structural properties of habitat networks. For the assessment of habitat connectivity, a distinction between structural and functional properties is commonly made. Taylor et al. (2006, p. 30) write that "structural connectivity [...] describes only physical relationships among habitat patches such as habitat corridors or inter-patch distances. [...] Functional connectivity, on the other hand, increases when some change in the landscape structure (including but not limited to changes in structural connectivity) increases the degree of movement or flow of organisms through the landscape." Here we apply this classification to both the edges (that express habitat connectivity) and the nodes in habitat networks. Especially important structural properties of habitat patches (i.e., nodes) are their size and their quality (Pascual-Hortal and Saura, 2006; Prevedello and Vieira, 2010; Hodgson et al., 2011). Influential structural properties of the edges in habitat networks are their geographic distance and the landscape between habitat patches (the latter is commonly referred to as matrix; Hodgson et al., 2011). Functional properties of the nodes in a habitat network are for instance the presence or abundance of certain species in habitat patches, whereas the existence or frequencies of movement among patches are functional properties of the edges.

Functional properties of habitat networks result from behavioral responses of species to their surroundings, which are generally specific to a (group of) species (Concepción et al., 2015). Therefore, separate habitat networks need to be parameterized for each (group of) species. Empirical studies have shown that changes to habitats and their surroundings can affect species richness (Fahrig, 2003) as well as the abundance and occurrence of species in habitats (Prugh et al., 2008;

Thornton et al., 2011). Habitat quality affects, for instance, patch occupancy (e.g., Thomas et al., 2001; Fleishman et al., 2002). Geographic distance as well as matrix composition and configuration between habitat patches have effects on the movement probability of organisms between patches (Holderegger and Wagner, 2008). Therefore, changes to the landscape matrix among habitat patches can have an effect on a patch's emigration and immigration rates (e.g., Todd et al., 2009). Also genetic processes can be influenced by habitat patch size and isolation (i.e., a measure calculated from a patch's geographic distance to other patches; e.g., Holmes et al., 2013). Through biological interactions between species, certain structural landscape changes also exert indirect effects on species. This is, for instance, shown in a study by Todd et al. (2008) where habitat disturbance led to an increase in fire ants, which in turn led to a decrease of their prey (i.e., amphibians) in these habitats. Also mathematical models of ecological processes in habitat networks frequently use the above structural properties and species characteristics as explanatory variables. For instance, metapopulation dynamics can be assessed from patch isolation, patch size and a species' migration range (Hanski, 1998). Several habitat connectivity indices are calculated from habitat size, habitat quality and the probability a species moves between habitat patches (which can be a function of matrix quality and interpatch distance; Saura and Pascual-Hortal, 2007).

The relative importance of the structural properties of habitat networks (i.e., habitat size, quality, isolation and matrix quality) on ecological processes is a subject of debate. From a quantitative review of 104 studies, Prevedello and Vieira (2010, p. 1205) concluded that "overall, the type of matrix is important, but patch size and isolation are the main determinants of ecological parameters in landscapes." In another review, Hodgson et al. (2011, p. 148) found that "variations in habitat area and quality have bigger effects [on population viability] than variations in spatial arrangement of habitats or properties of the intervening land." With respect to biodiversity, Fahrig (2013, p. 1655) posed that "the number of species in a patch is a function of both the size of the patch [...], and the area of habitat in the landscape surrounding the patch [...]." In a reaction to the latter publication, Hanski (2015) argues that the number of species is not only a function of habitat amount, but also of habitat fragmentation. Despite these mixed findings, patch size is the one variable that is consistently mentioned as being an important determinant for ecological processes.

Functional properties of networks (e.g., the presence or abundance of a species) can also influence the structural properties of habitat networks (**Figure 1**). Although the ability of plant and animal species to alter their surroundings is generally small compared to that of humans, the influence of organisms on their environment should not be neglected (Wright and Jones, 2006). So-called "ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they modify, maintain and create habitats" (Jones et al., 1994, p. 373). Ecosystem engineers are represented in many organismic groups from small organisms, such as ants or phytoplankton, to large ones, such as beavers, elephants and forest trees (Jones et al., 1994).

Influences of a Settlement Network on Habitat Networks

The influences of the processes taking place in settlement networks on those in habitat networks are manifold and studied in the research fields of road ecology and urban ecology. For comprehensive overviews of the findings in these fields, we refer to Forman et al. (2003), Hansen et al. (2005), Gaston (2010), Luck and Smallbone (2010), Forman (2014), Van der Ree et al. (2015) and Bennett (2017). The influence of a settlement network on habitat networks can be characterized as a "one-to-many" relationship (**Figure 1**), as changes in a settlement network will usually affect multiple species. For instance, in most cases road construction or changes to traffic flows will not only affect a certain species, but a range of species that are sensitive to such changes. Likewise, the conversion of forest patches to residential areas will affect all species inhabiting the respective patch to a certain degree.

Many changes to settlements will affect the structural properties of habitat networks. A direct effect is caused by the sealing of land that is used for constructing buildings and other urban structures, which renders the land uninhabitable for most species, thereby destroying habitat patches, reducing their size or fragmenting them (McKinney, 2002). However, within settlements the intensity of these effects is not homogenous in space and usually follows a gradient from densely built inner cities to relatively sparsely built peri-urban areas (i.e., commonly refered to as the "rural-urban gradient"; McDonnell and Hahs, 2008). For example, land-use change radiating out from settlement centers can produce a habitat-loss gradient, which is "a gradient of natural habitat loss that steepens from rural areas toward the urban center" (McKinney, 2002, p. 884). The shape of this rural-urban gradient strongly depends on the type of outward expansion that a city experienced in the past (Forman, 2014). A settlement's influence can also reach beyond its boundaries. Habitat quality surrounding settlements can be affected by, for instance, changes in nutrient and hydrological cycles and water quality, disturbance from domestic animals like dogs and cats, and disturbance from recreational activities by humans (Hansen et al., 2005). Land-use change in and around settlements can also replace natural land cover with land-uses that are less permeable for species (Bierwagen, 2007), thereby changing the structural properties of a habitat network's edges (i.e., inter-patch movement is a function of, among others, the edge's structural properties).

As with settlements, roads and traffic can also have profound effects on the structural properties of habitat networks. Apart from the habitat destruction directly caused by road construction (Seiler, 2003; Coffin, 2007), influences of roads on habitat networks usually radiate into the surrounding landscape, creating a "road-effect zone" (Forman, 2000; Coffin, 2007; Ibisch et al., 2016). Not only the habitat size, but also the habitat quality can be affected both at the location of roads and in their surrounding (Coffin, 2007). Factors like traffic noise, pollutants, light and invasive species can reduce habitat quality in the landscape surrounding a road (Spellerberg, 1998; Seiler, 2003; Hulme, 2009). Additionally, road construction often facilitates access of humans into the surrounding natural habitats, which can cause a range of disturbances, such as outdoor sports (Trombulak and Frissell, 2000). Roads and traffic can also change the structural properties of edges in a habitat network, by changing the permeability of the matrix between habitat patches (Jaeger et al., 2005; Holderegger and Di Giulio, 2010).

Changes to the settlement network can also directly affect the functional properties of habitat networks. In many cases, processes leading to habitat loss or degradation are also accompanied by the destruction or removal of plants and animals from the area. Worldwide, settlement expansion, for example, triggers the spread of non-native species and changes biotic interactions (McKinney, 2002; Hansen et al., 2005). Similar effects can also be found in the surroundings of new roads (Trombulak and Frissell, 2000). Avoidance of roads or traffic and mortality by cars can reduce species' movement between habitat patches or populations (Jaeger et al., 2005; Holderegger and Di Giulio, 2010). Mitigation structures, like wildlife over- or underpasses and fencing, have proven to be effective to reduce roadkill (Rytwinski et al., 2016), but conclusive evidence for positive effects on population connectivity at the landscape scale is rarely documented (Van der Ree et al., 2007; Corlatti et al., 2009).

Not all changes to the habitat networks resulting from changes in settlement networks are negative for biodiversity. The reaction of species to settlements ranges from species that adapt to such human surroundings to species that are very sensitive to any disturbance caused by humans (McKinney, 2002, 2008; Hansen et al., 2005; Reed et al., 2012; Concepción et al., 2015). Whereas urban core areas are generally found to be species poor (McKinney, 2002; Reed et al., 2012), suburbs can actually be relatively species rich (McKinney, 2008). However, the relatively high abundance of alien species in suburban areas can also explain—at least partially—the relatively high biodiversity in these areas. In Central Europe, the share of alien species in total species richness increases with city size (Pyšek, 1998). Also for roads, there are species that benefit from their existence as well as those that are deterred by them (Fahrig and Rytwinski, 2009; Benítez-López et al., 2010). Especially road verges present suitable habitats or movement corridors for certain species (Seiler, 2003). Road construction can thus also enhance the number of habitats or habitat connectivity for some species.

From this myriad of influences that settlement networks may have on habitat networks, there are two key factors that determine the severity of these effects at a local scale: the geographical location of settlements and roads relative to the habitats, and the density of people in settlements or using roads (Luck, 2007; Pautasso, 2007; Charry and Jones, 2009). The locations of roads and highways are necessary to determine those habitats that are influenced by road or settlement development. However, the magnitude of these influences is probably determined in large part by the intensity with which urban and peri-urban areas and roads are used by humans. Charry and Jones (2009, p. 159) argued that "road location and traffic volume are the two most important factors to assess when evaluating a road's potential impacts [on wildlife]." In addition, Luck (2007) stated that complex interactions of social, economic and demographic variables influence species diversity in and around settlements, but that human population density is likely a key driver of all these interactions. Although a positive correlation between human population density and species richness has repeatedly been found in studies at large spatial scales and with a large spatial grain, at regional scales and with a smaller grain (i.e., study area less than 10,000 km^2 and grain lower than 1 km^2) this correlation is generally negative (Pautasso, 2007). However, also at national scales McKee et al. (2013, p. 776) shows "that human population density is a key ultimate cause, and probably in many places a proximate cause, of species of mammals and birds becoming threatened with extinction." The suburban peak in biodiversity is probably due to the tolerable human disturbance and the large diversity of habitats and niches found in suburban areas (McKinney, 2002). Increasing population and building density (i.e., urban densification; see below) in suburban areas could thus reduce the number of species in these suburban habitats. In several studies, human population density surrounding nature reserves was found to be positively correlated with the number of extinctions of mammal species in these reserves (Brashares et al., 2001; Parks and Harcourt, 2002).

Influences of Habitat Networks on a Settlement Network

There are several potential influences of plants or animals on humans, but we here specifically focus on those aspects of habitat networks that, if changed, can bring about changes to the structural or functional properties of the settlement network (e.g., settlement sizes, population density, traffic flows, attractiveness of settlements or mobility). The influence of habitat networks on a settlement network will usually result from the combined effect of all habitat networks in an area (i.e., a "many-to-one" relationship between habitat networks and a settlement network; **Figure 1**). We have classified two kinds of effects of habitat networks on settlement networks: direct influences and influences resulting from the implemented governance system.

The direct effects of habitat networks on settlement networks are relatively limited. Some human communities in developing countries can be - to some degree - directly dependent on certain undomesticated species or groups of species for their livelihood (Díaz et al., 2006; CBD, 2010). Disappearance of these species thus threatens the existence of these communities, which could force them to relocate to other areas. In developed countries, however, livelihoods of most people do no longer directly depend on the existence of wildlife. Another direct effect between habitat networks and settlement networks could come from pathogens. Historically, settlement abandonments following disease outbreaks have been reported (McLeman, 2011), such as with the plague that was spread by fleas on rats throughout Europe (Hirschfeld, 2006). In modern times, the effect of such outbreaks will probably be less devastating, but can nevertheless have an impact on the habitability of certain regions for humans (e.g., Sachs and Malaney, 2002). The above two examples (i.e., dependence on wildlife for livelihood and changes in habitability due to disease) also reflect the few cases where there is a "one-to-one" relationship between habitat and settlement networks. Another potential direct effect of habitat networks on settlement networks is urban sprawl as one of the drivers for urban sprawl is the desire of many city inhabitants to move to the countryside (i.e., country-living desire; Bhatta, 2010). However, there is little evidence that desire is directly caused by a higher biodiversity or the occurrence of certain species in the countryside. Instead, the country-living desire is better explained by factors like green surroundings, spaciousness and high numbers of recreational opportunities in the countryside (Fernandez et al., 2005; Tobias et al., 2016). Vehicle-wildlife collisions are probably one of the few examples in which mobility of humans is directly affected by the mobility of animals. Although such collisions can cause human fatalities and significant economic damage (Huijser et al., 2009), to our knowledge there are no reports of significant reductions in traffic volumes following vehicle-wildlife collisions, unless mitigation measures are being taken (see below).

The majority of effects that habitat networks can have on a settlement network will be indirect via governance systems focussed at protecting nature and the environment. The way a governance system is shaped, depends on the "normative lens" through which decision makers and society view a certain problem and define their desired system states (**Figure 1**). Existing norms, principles and values "underpin all decisions since they inspire those who govern how to think and make judgments about how the world works and how to act in particular situations" (Kooiman and Jentoft, 2009, p. 818). In social-ecological systems, the norms and values about the relationship of humans with nature have effects on governance systems (Kooiman and Jentoft, 2009). Different environmental ethics, such as anthropocentrism, biocentrism or ecocentrism, are anticipated to result in different environmental policy measures (Stenmark, 2002).

There is a large variety of nature conservation measures that can be taken to improve or maintain the components of habitat networks (i.e., increasing the number and size of habitats or increasing their quality and their connectivity; Salafsky et al., 2008). These measures can affect settlement networks in several, sometimes unexpected, ways. For example, the establishment of a conservation area could increase the prices of surrounding residential areas (e.g., Lutzenhiser and Netusil, 2001). However, this effect may be less strong if accessibility to the open space is not guaranteed (Geoghegan, 2002). Restricting accessibility to open spaces can be a potential conservation measure to reduce human disturbance in these areas (Newsome et al., 2013). To compensate or off-set the potential damage people are causing by developing housing in natural surroundings, economic conservation measures can be implemented, such as cap-and-trade, subsidy or tax systems and payments for ecosystem services (Pirard, 2012; Barrett et al., 2013). In order to preserve natural habitats under a growing human population, one of the most often proposed solutions is urban densification (i.e., increasing the density of buildings in existing settlements; Jabareen, 2006; Gaigné et al., 2012; Tobias et al., 2016). Also to mitigate the negative effects that roads and traffic can have on habitat networks there exist a range of potential conservation measures (Keller et al., 2003), some of which have effects on traffic flows in the settlement networks. By reducing vehicle speeds or closing roads during certain hours of the day (i.e., traffic calming), road avoidance by animals or the number of wildlife-vehicle collisions can be reduced, but at the same time traffic volumes on other roads are increased (Van Langevelde and Jaarsma, 2009). Conservation measures can thus be seen as the proximate causes of many changes in settlement networks as a reaction to changes in habitat networks.

IMPLEMENTING MODELS OF COUPLED HABITAT AND SETTLEMENT NETWORKS

Several authors have developed models that can be considered predecessors of models that couple habitat and settlement networks (e.g., Jaeger et al., 2006; Jaeger, 2007; Van Langevelde and Jaarsma, 2009; Rhodes et al., 2014; Van Strien and Grêt-Regamey, 2016). For instance, Jaeger (2007) simulated connectivity in landscapes with different road configurations. Similarly, Rhodes et al. (2014) modeled Koala movement and mortality in a real landscape and derived how mortality would change when increases in traffic volume were either assigned to existing roads or divided over new roads. In these studies, traffic volumes were used as a proximate variable for the investigated ecological process. However, changes in traffic flows were not modeled dynamically. In contrast, Van Langevelde and Jaarsma (2009) modeled the effect of the size of traffic calmed areas (i.e., areas in which the number of cars is reduced) on animal population persistence. In their study, traffic flows were dynamically modeled based on the size of the traffic calmed area. Yet, human population density was equal throughout the simulated landscapes. Recently, Van Strien and Grêt-Regamey (2016) simulated habitat connectivity in landscapes in which both the configuration of the road network and that of the settlements were varied. Traffic flows were calculated from human population sizes in the settlements and the travel times between settlements. The above studies showed that there are significant effects of settlement and road configuration on ecological processes taking place in habitat networks. Nevertheless, they do not adhere to all the recommendations for models of coupled settlement and habitat networks that we have outlined below.

Recommendations for Models of Coupled Settlement and Habitat Networks

Based on the description of the conceptual framework in the previous sections and its depiction in **Figure 1**, we have formulated four recommendations for the modeling of coupled habitat and settlement networks.

(1) *Both networks should be spatially embedded.* The settlement and habitat networks are by definition spatial networks, but the interactions between the networks are also of a spatial nature. For instance, the expansion of a settlement will mainly have an effect on the habitats surrounding this

settlement. Similarly, increases in traffic will mainly have an influence on the quality of habitats within a road's effect zone. For many ecological processes taking place in habitat networks, habitat patch size is an important determinant (see section Interactions Within Habitat Networks). For these reasons, it is important that both networks are embedded in a spatial plane (or landscape) in which the effects that the networks have on each other can be simulated. The competition for space between the two networks can be made spatially explicit, by altering the locations and geometries of settlements (e.g., area), roads, habitats and other landscape elements over time. These locations and geometries are important variables in defining the structural properties of the settlement and habitat networks.

(2) *Multiple habitat networks should be modeled for a variety of species*. The "many-to-one" influence of habitat networks on settlement networks (**Figure 1**) makes it necessary to develop habitat networks for multiple species in order to get an accurate representation of biodiversity. Ideally, habitat networks are constructed for every species in the study region, but this is an extremely laborious task. It is therefore advisable to select (groups of) focal species so that the range of possible responses to and influences on settlement networks is covered (Van Teeffelen et al., 2012). For instance, focal species can be selected from different dispersal guilds that contain species with similar movement characteristics and habitat requirements (Lechner et al., 2017). Focal species can also be selected based on their functional traits (De Bello et al., 2010), which describe the role that a group of species play in ecosystem functioning or in the provision of ecosystem services (Díaz et al., 2007; Luck et al., 2009). In order to ensure multifunctional ecosystems, it is important that as many functional traits as possible are represented in an ecosystem and thus included in the modeling.

(3) *Influences between the networks should be in both directions*: i.e., from settlement to habitat networks and vice versa. Only if both networks react to changes in the other network, can a dynamic feedback mechanism be simulated. Abrupt critical transitions especially occur in systems with positive feedbacks (Angeli et al., 2004; Scheffer et al., 2012). Such feedbacks can also take place through interactions within one of the networks. Therefore, even a one-way influence from one network can trigger an abrupt transition in the other network. Nevertheless, if influences between the networks are not considered in both ways, the effects of this transition will stay limited to the network in which it takes place and a full picture of its effects in the social-ecological system cannot be obtained. In order to incorporate the influence of habitat networks on settlement networks in models, it is advisable that the latter is capable of reacting to spatial or aspatial nature conservation measures, which we have identified as the main influence of habitat networks on a settlement network (see section Influences of Habitat Networks on a Settlement Network).

(4) *Both the structural and functional properties of networks should be modeled*. In both networks, changes to the structural properties usually have an effect on their functional properties (section Interactions Within Settlement Networks and Interactions Within Habitat Networks). Especially in settlement networks, this effect is also vice versa (section Interactions Within Settlement Networks). In many cases, changes to functional properties in one network can trigger changes to structural properties in the other network. For example, we have identified human population density in the settlement network (i.e., a functional property) as one of the most influential drivers of changes in habitat network (see section Influences of a Settlement Network on Habitat Networks). However, the resulting changes in the habitat network are often to its structural properties (e.g., decreases in habitat quality due to an increase in traffic). Likewise, following reductions in the occurrences of species (i.e., a functional property) in multiple habitat networks (i.e., biodiversity loss), nature conservation measures can be implemented, which can have an impact on both the structural and functional properties of the settlement network (see section Influences of Habitat Networks on a Settlement Network).

Modeling Settlement Networks

Following the above recommendations, both the structural and functional properties of settlement networks as well as their interactions are ideally included in the model of the settlement network. Furthermore, the model should be spatially explicit at a local scale and predict local human population density and traffic flows as key variables. Particularly useful for this purpose are land-use transport interaction (LUTI) models, "which explicitly model the two-way interaction between land use and transport to forecast the likely impacts of land-use policies [. . .] and of transport policies [. . .]" (Wegener, 2014, p. 742). Although many different LUTI models have been developed based on various theoretical assumptions and which are applicable to various spatial scales and aggregation levels (Wegener, 2014; Acheampong and Silva, 2015), many of them aim to simulate the interactions between the choices people make regarding transport and the locations where they reside, work and go to for other activities such as recreation (e.g., see the conceptual model of a land-use-transport system in Acheampong and Silva, 2015). Wherever attractiveness or demand increases, construction of new buildings or roads will take place (e.g., see the land-use transport feedback cycle in Wegener and Fürst, 1999), provided that this is permitted under the implemented land-use or transport regulations. By changing the regulations, LUTI models can also be used to forecast the effect of policy measures on functional properties of a settlement network. This has, for instance, been shown by Bodenmann et al. (2014), who used a Swiss-wide LUTI model to predict how population sizes in municipalities may change due to changes in land-use regulations or changes in the transport infrastructure. In a similar way, LUTI models could also be used to forecast the effects of alternative environmental governance systems. A further advantage of LUTI models, is that many of them are agent-based models (Wegener, 2014; Acheampong and Silva, 2015), which are considered particularly useful when modeling social-ecological systems, as they allow simulating the influence of the decisions of individuals on a system's dynamics (Milner-Gulland, 2012). From several points of view, LUTI models are thus suitable for modeling the interactions in settlement networks.

A drawback of many current LUTI models is that "land-use" in these models usually refers to the built environment and that they are thus not capable of simulating changes in land-use and human influences that radiate from settlements and roads. Combining LUTI models with land-use models that consider a broader spectrum of land-uses (e.g., Verburg and Overmars, 2009) is a potential approach to simulate such gradients. Especially spatially-explicit models that link human population density to land-use change (e.g., Verburg et al., 1999) can prove useful for this purpose. To our knowledge, no models have been developed that specifically model the effects of roads or traffic on surrounding habitats.

Modeling Habitat Networks

As with the settlement networks, the habitat network models should capture the interactions between the structural and functional properties of the networks. Agent-based models can also be used to simulate habitat usage and movement of individual animals throughout a landscape (Tang and Bennett, 2010). For example, Rhodes et al. (2014) used an agent-based model to simulate koala movement through a landscape and assess how the survivability of Koalas was affected by traffic. Although such agent-based models are capable of capturing complex behaviors of organisms in realistic and heterogeneous landscapes (Wallentin, 2017), they are usually very data hungry and laborious to program and parameterize. The latter becomes problematic when one has to setup models for multiple species (see section Recommendations for Models of Coupled Settlement and Habitat Networks). An alternative to agent-based models of habitat networks are aggregated network-based models (Urban et al., 2009; Galpern et al., 2011). In these models, discrete patches of habitat need to be delineated to form the nodes in the habitat network, which is a simplification of the heterogeneous landscapes that can be used in agent-based models. The edges in the network can be weighted based on, for instance, the permeability of the matrix or on species characteristics (e.g., dispersal kernels). From these network-based models a variety of network measures can be calculated that express the importance of nodes or edges in the network or the connectedness of the network as a whole (Rayfield et al., 2011). These network measures can be used to predict the occurrence of species in habitat patches (e.g., Pereira et al., 2011) or the diversity in certain groups of species (e.g., for amphibians; Ribeiro et al., 2011). Both agent-based and network-based models require information on habitat and movement characteristics of (groups of) species. Although it is laborious to collect such data in the field for multiple species, trait databases that compile such data for certain taxa are increasingly being developed (e.g., for amphibeans; Trochet et al., 2014).

Both agent-based and network-based models can thus capture the effect of the structural properties of habitat networks on its functional properties. However, the opposite effect (i.e., functional on structural properties) remains difficult to model. In the relatively young field of study of ecosystem engineers, some models have been developed, but most are mainly conceptual and not operative (Wright and Jones, 2006; Hastings et al., 2007). The limited number of general models of ecosystem engineers currently reduces the possibilities to capture the feedback of functional on structural habitat network properties.

Coupling of Settlement and Habitat Networks

The actual coupling of the spatially-explicit settlement and habitat network models can be achieved by ensuring that output variables from the settlement network model function as the input variables for the habitat network model and vice versa. Edge weights in habitat networks can include the presence of transportation infrastructure (Pereira et al., 2011) or traffic intensity on roads (Scolozzi and Geneletti, 2012). For instance, in Van Strien and Grêt-Regamey (2016), traffic volumes were output of the settlement network model. These traffic volumes were subsequently input variables for the habitat network model and used to calculate habitat connectivity for several animal species. Modeling the effect of landscape and human mobility changes on habitat connectivity can be operationalized with resistance surfaces (Zeller et al., 2012; Cushman et al., 2014). Although these surfaces are usually static, they can also be modeled in a dynamic way so that they react to changes in the settlement network (e.g., Van Strien and Grêt-Regamey, 2016). The influence of habitat networks on settlement networks can be implemented in a similar way: output of the habitat network models (e.g., the loss of a species or a reduction in biodiversity) can trigger certain nature conservation measures, which are simulated by adjusting input variables of the settlement network model (e.g., density of housing or capacity of certain roads). By iteratively performing this information exchange between the settlement network and habitat network models, the dynamics of this social-ecological system can be simulated over time.

A challenge in simulating such time series are the differences in temporal scales of the processes taking place in the networks. For example, settlement development takes place at a much slower pace than changes to human travel behavior (Wegener and Fürst, 1999; Levinson, 2008). This means that a conservation measure like traffic calming will have an immediate effect on the traffic volumes in and around the traffic calmed areas, but that the resulting changes to settlement sizes or human population densities will only become visible over a longer time period. Such temporal lags should be considered when determining the period over which the dynamics in coupled settlement and habitat networks are simulated.

APPLICATIONS OF COUPLED SETTLEMENT AND HABITAT NETWORK MODELS FOR BIODIVERSITY CONSERVATION

Once a model of coupled settlement and habitat networks has been developed, we envisage several potential applications of such models to benefit biodiversity conservation. Our overview of potential applications of coupled settlement and habitat network models is not exhaustive, but is illustrative of the breadth or research topics in which such models can be applied.

Complex Networks Theory

Models of coupled settlement and habitat networks can draw from and contribute to the rapidly increasing fundamental research on complex networks. Many complex networks are represented as coupled networks (also called multiplex, multi-layered or interdependent networks or network of networks; Gao et al., 2014). In coupled networks, Buldyrev et al. (2010) have shown that small changes in one network can bring about a cascade of changes in both networks leading to abrupt fragmentation of the coupled system. Coupled spatial networks, such as coupled settlement and habitat networks, appear especially sensitive to small changes in either of the networks (Bashan et al., 2013). Such abrupt changes usually only take place beyond certain thresholds. These thresholds are influenced by properties of the coupled spatial networks, such as the fraction of interdependent nodes (Bashan et al., 2013) or the maximum geographic distance between nodes (Danziger et al., 2014). Identification of such thresholds and associated network properties is thus very important for vulnerability assessments of coupled networks, such as coupled settlement and habitat networks. However, knowledge of many theoretical studies on coupled spatial networks is not directly transferable to coupled settlement and habitat networks, as the nature of the dependencies between the coupled networks is different. In many studies, the networks are coupled by dependencies between spatially coinciding nodes in either networks (e.g., Buldyrev et al., 2010; Bashan et al., 2013; Danziger et al., 2014). In coupled settlement and habitat networks, the nodes are usually not spatially coinciding (i.e., settlements and habitats are spatially separated) and the dependencies between the networks are not only between nodes, but also between edges (e.g., roads intersecting animal movement paths) and between nodes and edges (e.g., habitat quality affected by traffic; Van Strien and Grêt-Regamey, 2016). Models of coupled settlement and habitat networks, in which these dependencies are incorporated, could thus lead to new insights in the behavior of coupled spatial networks and could potentially be used to assess the threat of rapid biodiversity loss in coupled settlement and habitat networks.

Resilience of Coupled Settlement and Habitat Networks

Analysis of the temporal dynamics in coupled settlement and habitat networks can also be used to estimate the resilience of this social-ecological system and all of its components (including biodiversity; Folke, 2006). The concept of system resilience was coined by Holling (1973, p. 17), who applied it to ecological systems and defined it as "a measure of the ability of these systems to absorb changes of state variables, driving variables, and parameters, and still persist." Later, the concept was introduced to social-ecological systems (a history of the concept is described in Folke, 2006). Nowadays there are many definitions of resilience as well as approaches to assess the resilience of a system (Hosseini et al., 2016). Resilience has been the focus of studies on habitat networks (e.g., Uden et al., 2014) as well as on settlement networks (e.g., Ip and Wang, 2011) and recently has also been introduced to complex network theory (e.g., Gao et al., 2016). Models of coupled settlement and habitat networks can potentially be used to determine how the system recovers after sudden exogenous or endogenous disruptions and thereby assess system resilience.

Models of coupled settlement and habitat networks can also be used to aid practitioners in adapting their management strategies or transforming their system to ensure the resilience in real settlement and habitat networks (Levin et al., 2013). Rapidly changing environment and land-use has led to the realization that habitat networks should not be managed as static entities, but as dynamic systems that are constantly changing (Van Teeffelen et al., 2012). For this reason, "adaptive governance" is often propagated (Chaffin et al., 2014). For adaptive governance, a broad range of management options should be kept open to be able to react to unexpected events and changing conditions. By analysing different socio-economic, environmental or demographic development scenarios, models of coupled settlement and habitat networks can help identifying management options that could become necessary in the future. These analyses can give insights into the overall resilience of the system of coupled settlement and habitat networks and shed light on the long-term survivability of specific species or biodiversity in general.

Conservation, Transport, and Urban Planning

Models of coupled settlement and habitat networks can also be used to aid conservation, transport and urban planning. In habitat networks, the importance of nodes or edges for network connectivity can be assessed (Galpern et al., 2011). Comparable analyses can also be performed in settlement networks (e.g., Jenelius and Mattsson, 2015). These analyses are usually performed with node or edge removal experiments. Although such experiments can be applied for the analysis of resilience in networks (see section Resilience of Coupled Settlement and Habitat Networks), they can also serve a more applied goal: to prioritize spatial planning. For instance, Pereira et al. (2017) constructed habitat networks for 20 bird species in North-East Spain and ranked habitat patches based on their importance for network connectivity in order to prioritize nature conservation actions. With models of coupled settlement and habitat networks, the importance of nodes or edges in either network can be assessed based on current or forecasted changes in the other network. For example, by assessing which edges in habitat networks are most affected by current or future traffic on intersecting roads, optimal locations for roadkill mitigation measures can be identified (Loro et al., 2015; Mimet et al., 2016; Rytwinski et al., 2016). Especially when such mitigation measures can have an effect on traffic flows, such as traffic calming (Van Langevelde and Jaarsma, 2009), is it important to not only assess the effects of the measure on the habitat network but also its effect on the settlement network. Another example of a conservation and urban planning measure that can potentially have effects on both the settlement as well as the habitat networks is urban densification, which is promoted for reducing the urban land consumption and commute distances (Gaigné et al., 2012). However, on the long term urban densification can bring about significant changes in the settlement network, which can have unforeseen negative environmental effects (Gaigné et al., 2012). Models of coupled settlement and habitat networks could help identifying these negative effects and adapt conservation, transport and urban planning accordingly.

CONCLUSIONS

In this article, we present coupled settlement and habitat networks as a social-ecological system and promote the development of models of such coupled networks to generate new insights and effective policy for biodiversity conservation. In our conceptual framework of models of coupled settlement and habitat networks, we have described the network-internal interactions as well as the influences between the networks. In summary, there are strong two-way interactions between the structural properties of a settlement network and its functional properties (**Figure 1**). Whereas, in habitat networks, structural properties mainly influence their functional properties (**Figure 1**). Through ecosystem engineer species, a habitat network's functional properties in some cases can also influence its structural properties. Changes to settlement networks can have direct as well as indirect influences on the structural and functional properties of habitat networks (**Figure 1**). For both settlements and roads, these influences are not limited to only the location of a settlement or road, but also radiate out into their surrounding landscape. Direct influences of habitat networks on settlement networks are usually limited. More prevalent are indirect influence caused by nature conservation measures that are implemented to maintain or enhance species or biodiversity. The governance system to which these conservation measures belong, is influenced by the normative lens through which society views human-nature relationships (**Figure 1**).

Due to these numerous network-internal interactions in settlement or habitat networks and the between-network influences, it is difficult to assess whether changes to any part of either network can result in positive or negative feedbacks leading to complex system behavior. Coupling models of settlement networks and habitat networks in a dynamic way, can reveal such complexities that cannot be found when only parts of the social-ecological system are studied. These models do not have to be built from scratch, but can be based upon existing models of settlement or habitat networks, of which we have presented some in this article (e.g., habitat network models or land-use transport interaction models). Nevertheless, new data may have to be collected to parameterize some of the relationships between or within the networks. We see several potential applications of models of coupled settlement and habitat networks for biodiversity conservation, ranging from fundamental research on desirable network properties to more applied assessments of weaknesses in existing settlement or habitat networks for conservation planning purposes. We envisage that integrated models of coupled settlement and habitat networks can contribute to a world in which both human well-being is ensured and biodiversity is maintained.

AUTHOR CONTRIBUTIONS

The conceptual model presented in this manuscript is the result of a series of discussions and meetings that took place as part of the CHECNET (Coupling human and ecological networks for sustainable landscape and transport planning) project. All authors are part of this project, have participated in the meetings and discussions and have contributed to the formulation of the conceptual model. MvS wrote a first draft of the manuscript, which was reviewed by KA, ID, AG, AG-R, AK-M, DO-R, and RH.

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